Identification of Peroxisome Proliferator-Activated Receptor Alpha (PPARα)-Dependent Genes Involved in Peroxisome Proliferator-Induced Hepatocarcinogenesis

LEUNG Wan-chi

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of
Master of Philosophy
in
Environmental Science Programme

Nov 2005

Thesis Committee

Professor C.C.Wan (Chair)

Professor S.S.T. Lee (Thesis Supervisor)

Professor C.C. Wan (Committee Member)

Professor Y.Y. Ho (Committee Member)

Professor T. Pineau (External Examier)

Abstract

Peroxisome proliferator-activated receptors (PPARs) are the members of the steroid/retinoid nuclear receptor family. PPARs include three isoforms: α, δ/β, and γ. PPARα is the isoform responsible for lipid metabolism and peroxisome proliferator (PP)-induced pleiotropic responses and hepatocarcinogenesis. Short-term (2 weeks) administration of PP causes peroxisome proliferation, hepatomegaly and induction of gene expressions that involved in fatty acid β-oxidation. Long-term (11 months) administration of PP causes hepatocarcinogenesis. PP is a group of chemicals include some plasticizers such as DEHP di(2-ethylhexyl)phtalate) and hyperlipidemic drugs such as clofibrate and Wy-14,643. As humans are exposed to peroxisome proliferators chronically, the long-term effect of peroxisome proliferators on human should be identified.

In my study, Wy-14,643 compound was selected as 100% hepatocarcinogenic effect was observed after long-term treatment. PPARα wild-type and PPARα (-/-) mice were fed with a control or a 0.1% Wy-14,643 diet for 11 months in order to find out the genes that are PPARα dependent and Wy-14,643 responsive. In order to study the time-dependent liver damage and genes expression that are induced by Wy-14,643, 24 hours gavage feeding, 1 week, 2 weeks and 6 months feeding experiments were also performed.

Hepatomegaly was observed in the PPARα (+/+) fed with 0.1% Wy-14,643 after 1 week treatment and the level of liver enlargement was increased with prolonged period of treatment. The relative organ weights of white fat, brown fat and spleen in the 0.1% Wy-14,643 fed PPARα mice were lower compared to their controls. Serum cholesterol and triglyceride levels were also measured for different time points. Serum cholesterol level was significantly higher in 0.1% Wy-14,643 fed PPARα (+/+) mice at 11 months treatment. Time-dependent decrease in serum triglyceride level was observed in PPARα (+/+) mice fed with 0.1% Wy-14,643. The time-dependent decrease was started at 24 hours gavage feeding and continued to 11 months treatment.

Histology was performed to study the liver damage that induced by Wy-14,643 after chronic exposure. Irregular hepatocytes arrangement, enlarged sinusoidal spaces and trabecular cell clusters were observed in PPARα (+/+) mice fed with 0.1% Wy-14,643. In PPARα (-/-) ice, with the disruption of PPARα gene, lipid accumulation was found in both control and 0.1% Wy-14,643 treatment group started at 24 hours gavage feeding.

After 11 months feeding experiment, fluorescent differential display was employed to screen out the genes in liver that are PPARα–dependent and Wy-14,643 responsive as fluorescent differential display is able to compare several treatment groups at the same time. A total of eleven fluoroDD gels were performed and

seventeen PPARα–dependent and Wy-14,643 responsive genes were confirmed in this project. They include peroxisomal delta 3, delta 2-enoyl-Coenzyme A isomerase, mouse cysteine sulfinic acid decarboxylase, mouse acetyl-Coenzyme A dehydrogenase, medium chain, peroxisome biogenesis factor 16, mouse Cyp4a14, mouse peroxisomal bifunctional enzyme, mouse Cyp4a10, very-long-chain acyl-CoA synthetase, mouse peroxisomal acyl-CoA oxidase, mouse hydroxysteroid (17-beta) dehydrogenase 11, mouse adipose differentiation related protein and mouse carnitine O-octanoyltransferase were confirmed to be up-regulated by 0.1% Wy-14,643 and PPARα-dependent. Mouse apolipoprotein A-V, mouse UDP-glucuronosyltransferase 2 family member 5, mouse serine protease inhibitor 1-1, mouse major urinary protein II and mouse carboxyesterase were confirmed to be down-regulated by 0.1% Wy-14,643 and PPARα dependent.

Temporal expression of all the seventeen genes (except mouse apolipoprotein A-V) was also performed to observe the time-dependent changes of these genes expression. Up-regulation of mouse peroxisomal delta 3, delta 2-enoyl-Coenzyme A isomerase, mouse acetyl-Coenzyme A dehydrogenase, medium chain, peroxisome biogenesis factor 16, mouse Cyp4a14, mouse peroxisomal bifunctional enzyme, mouse Cyp4a10, very-long-chain acyl-CoA synthetase, mouse peroxisomal acyl-CoA oxidase, mouse hydroxysteroid (17-beta) dehydrogenase 11, mouse adipose differentiation

related protein and mouse carnitine O-octanoyltransferase were started at 24 hours and persisted to 11 months treatment while up-regulation of mouse cysteine sulfinic acid decarboxylase was started at 1 week and persisted to 11 months. Down-regulation of mouse serine protease inhibitor 1-1, mouse major urinary protein II and mouse carboxyesterase was started at 1 week and continued to 11 months. Mouse UDP-glucuronosyltransferase 2 family member 5 was down-regulated at 2 weeks and persisted to 11 months.

Based on the genes identified, oxidative stress is suggested to be the mechanism of PP-induced hepatocarcinogenesis. However, we still not know whether other mechanisms are involved in the PP-induced hepatocarcinogenesis. It is suggested that PP-induced hepatocarcinogenesis is due to the combined effect of oxidative stress and inhibition of apoptosis and increase in cell replication. Further studies are necessary to find out the relationship of different mechanisms and the chronic effect of peroxisome proliferators on humans.

摘　要

過氧化物酶增殖因數啓動受體（PPARs）是類固醇/維生素 A 核受體家族的成員。PPARs 有三種亞型，α、δ/β和γ。其中 PPARα是參與脂肪代謝、過氧化物酶增殖因數（PP）誘導的多向應答以及肝臟腫瘤發生的主要亞型。短期（2 周）的 PP 處理會引發過氧化物酶體增殖，肝腫大以及誘發參與脂肪酸β氧化的基因的表達。長期（11 個月）的 PP 處理會導致肝臟腫瘤的發生。PP 是一類化學藥品包括一些可塑劑例如 DEHP 和高脂血藥物例如安妥明和 Wy-14,643。由於人類暴露於充滿過氧化物酶增殖因數的環境中，所以有必要研究過氧化物酶增殖因數對人類長期的影響。

在本研究中，主要研究 Wy-14,643 化合物的作用，因爲目前已知 Wy-14,643 的長期處理必然會引發肝臟腫瘤。用含有 0.1% Wy-14,643 的飼料和不含有 Wy-14,643 的常用飼料喂養 PPARα野生型和 PPARα敲除的小鼠，從而分離 PPARα依賴以及 Wy-14,643 誘導的基因。爲了研究肝臟損傷的時間性以及 Wy-14,643 誘導的基因表達，分別在喂養 24 小時、1 周、2 周以及 6 個月收集樣品進行試驗。

PPARα野生型的小鼠，在用含有 0.1% Wy-14,643 的飼料喂養 1 周後，發生肝腫大；隨著喂養時間的增加，肝腫大的程度也增加。同時，0.1% Wy-14,643 喂養的 PPARα野生小鼠，白脂肪、棕脂肪以及脾臟的重量比正常喂養的 PPARα野生小鼠要輕。本研究也檢測了不同時間點的小鼠血清中膽固醇和甘油三酯的水平。0.1% Wy-14,643 喂養了 11 個月

的 PPARα野生小鼠的血清中的膽固醇水平顯著的升高。而在 0.1% Wy-14,643 喂養的 PPARα野生小鼠中，從 24 小時起，血清中甘油三酯的水平隨著時間延長而降低。

採用組織學的方法，本研究檢測了長期 Wy-14,643 處理下，小鼠的肝臟損傷。在 0.1% Wy-14,643 喂養的 PPARα野生小鼠中，觀察到肝實質細胞的不規則排列，竇狀隙擴大以及橫紋細胞簇。而在 PPARα敲除小鼠中，由於缺失了 PPARα基因，不管是餵食常用飼料或者含有 0.1% Wy-14,643 的飼料，從 24 小時起，都觀察到脂肪堆積的情況。

在喂養了 11 個月後，採用熒光差異顯示（FluoroDD）技術從小鼠的肝臟中篩選 PPARα依賴以及 Wy-14,643 誘導的基因。採用此技術是因爲熒光差異顯示技術可以同時比較不用喂養組別的樣品。本研究從 11 塊 FluoroDD 膠中分離到 17 個 PPARα依賴及 Wy-14,643 誘導的基因。其中包括：過氧化物Δ-3, Δ-2-烯酰輔酶 A 異構酶, 小鼠半胱次磺酸脫羧酶, 小鼠乙酰輔酶 A 脫氫酶, 過氧化物生物合成因子 16, 小鼠細胞色素 4a14, 小鼠過氧化物雙功能酶, 小鼠細胞色素 4a10, 超長鏈酰基輔酶 A 合成酶, 小鼠過氧代物酰基輔酶 A 氧化酶, 小鼠輕基類固醇 (17-β) 脫氫酶 11, 小鼠脂肪分其化相關蛋白和小鼠肉碱O-辛酰輕移酶是 PPARα依賴以及 Wy-14,643 誘導上調的；而小鼠載脂蛋白 A-V,小鼠 UDP-葡糖醛酸轉移酶 2 家族成員 5, 小鼠絲氨酸蛋白酶抑制因子 1-1, 小鼠主要尿蛋白 II 和小鼠羧酶酯酶是 PPARα依賴以及 Wy-14,643 誘導下調的。

除了小鼠載脂蛋白 A-V, 本研究檢測了所有 16 個基因隨 Wy-14,643 處

理時間的表達水平變化。過氧化物Δ-3, Δ-2-烯酰輔酶 A 異構酶, 小鼠乙酰輔酶 A 脫氫酶, 過氧化物生物合成因子 16, 小鼠細胞色素 4a14, 小鼠過氧化物雙功能酶, 小鼠細胞色素 4a10, 超長鏈酰基輔酶 A 合成酶, 小鼠過氧代物酰基輔酶A氧化酶, 小鼠輕基類固醇 (17-β) 脫氫酶 11, 小鼠脂肪分其化相關蛋白和小鼠肉碱 O-辛酰輕移的表達水平從 Wy-14,643 處理 24 小時開始升高，一直持續到 11 個月；而小鼠半胱次礦酸脫羧酶的上調從 1 周持續到 11 個月。小鼠絲氨酸蛋白酶抑制因子 1-1, 小鼠主要尿蛋白 II 和小鼠羧酶酯酶表達水平的降低從 1 周開始，直至 11 個月；小鼠 UDP-葡糖醛酸轉移酶 2 家族成員 5 的下調從 2 周到 11 個月。

基於鑒定的基因，PP 誘導的肝臟腫瘤發生的機制可能是由於氧化作用。而然，我們不能確定是否還有其他機制參與 PP 誘導的肝臟腫瘤發生。本研究顯示，PP 誘導的肝臟腫瘤發生是氧化壓力、細胞調亡被抑止和細胞複製的增加的綜合作用。我們需要進行更深入的研究來檢測不同機制之間的關係及過氧化物酶增殖因數對人體的長期效應。

Acknowledgements

I would like to give my special thanks for my supervisor, Prof. Lee Sau-Tuen Susanna, for her support throughout these years. Her critical advice guidance on my project will be very useful in my future.

I would like to express my gratitude to Prof. Cheung Wing-Tai for his advise on my project and Prof. Chan Woo-Yee Woody for providing training on histology.

I am grateful to my colleagues Ms. Chen Ka-Pik, Ms. Li Sui-Mui, Ms. Lo Kam-Chun, Mr. Ng Lui, Ms. Sreedevi Avasarala and Ms. Sun Yan for their support and valuable advice in my research. Technical support from Mr. Kam Yiu-Wing and Mr. Chui Shun-Keung is also important for my project. I would also want to give my thanks for Ms. Chan Pui-Ting, Ms. Yuen Yee-Lok and Ms. Yau Wing-Yiu for their kind help.

Last but not least, I am extremely thankful to my parents and sisters for their support and encouragement during these years.

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List of Abbreviations

α2U	α2- urinary globulin
A	Absorbance
ADRP	Adipose differentiation related protein
ALDH	Aldehydehydrogenase
AP	Anchored primer
ApoA1	Apolipoprotein A-I
ApoA2	Apolipoprotein A-II
ApoA5	Apolipoprotein A-V
ApoAIII	Apolipoprotein C-III
ARP	Arbitrary primer
Asl	Argininosuccinate lyase
ATP	Adenosine triphosphate
ATT	α-1 antitrypsin
AOX	Acyl-CoA oxidase
BCIP	5-bromo-4-chloro-3-indolyl-phosphate
BLAST	Basic Local Alignment Search Tool
Bp	Basepair
BOX	Branched–chain acyl-CoA oxidase
bw	Body weight
CAT	Carnitine acetyltransferae
CAD	Coronary artery disease
cDNA	Complementary deoxyribonucleic acid
cm	Centimeter
Cideb	Cell death-inducing DNA fragmentation factor
CoA	Coenzyme A
Crot	Carnitine O-octanoyltransferase
CPT	Carnitine palmitoyltransferase
Csad	Cysteine sulfinic acid decarboxylase
CTL	Control diet
Cyp2a5	Cytochrome P450, family2, subfamily a, polypeptide 5
Cyp4a10	Cytochrome P450, family 4, subfamily a, polypeptide 10
Cyp4a14	Cytochrome P450, family 4, subfamily a, polypeptide 14
DBD	DNA-binding domain
DD	Differential display
DEHA	Di(2-ethylhexyl)adipate

DEHP	Di(2-ethylhexyl)phtalate
DNA	Deoxyribonucleic acid
DNTP	Deoxyribonucleotide-5-phosphate
DR-1	Direct repeat 1
DTT	Dothiothreitol
EPHX	Epoxide hydroxide
EDTA	Ethylenediaminetetraacetic acid
FDD	Fluorescent differential display
g	Gram
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GDP	Glycerol-3-phosphate and adenosine-5'-adiphate
HDL	High density lipoprotein
H_2O_2	Hydrogen peroxide
2-HPCL	2-hydroxyphtanoyl-CoA lyase
hr	Hours
Hsd17b11	Hydroxysteroid (17-beta) dehydrogenase 11
IPTG	Isopropyl-β-D-thiogalactoside
LBD	Ligand binding domain
LPL	Lipoprotein lipase
Kb	Kilobase
KO	Knockout
MCAD	Acetyl-coenzyme A dehydrogenase, medium chain
µl	Microlitre
µm	Micrometer
µM	Micromolar
µm	Micrometer
min	Minutes
mg/dl	Milligram/deci litre
ml	Millilitre
ml/g	Millilitre/gram
mM	Mililimolar
mo	Months
MUP II	Mouse major urinary protein II
NaCl	Sodium chloride
NBT	Nitroblue tetrazolium chloride
Ntanl	N-terminal Asn amidase
Ng	Nanogram
nm	Nanometer

orm 1	Orosomucoid 1
Oligo(dT)	Oligopolydeoxythymidine
PBFE	Peroxisomal bifunctional enzyme
PCR	Polymerase chain reaction
Peci	Peroxisomal delta3, delta2-enoyl-coenzyme A isomerase
Pex16	Peroxisome biogenesis factor 16
PP	Peroxisome proliferator
PPAR	Peroxisome proliferator-activated receptor
PPRE	Peroxisome proliferator responsive element
PUFA	Polyunsatruated fatty acid
RNA	Ribronucleic acid
Rnase 4	Ribonuclese 4
rpm	Round per minute
RT-PCR	Reverse transcription-polymerase chain reaction
RXR	Retinoid X receptors
Sac1 gene	Suppressor of actin mutations
SDS	Sodium dodecyl sulfate
sec	Second
Serpinf 2	Serine (or cysteine) proteinase inhibitor, clade F, member 2,
SPI	Serine (or cysteine) proteinase inhibitor, clade A, member 1a
Spi2	Serine (or cysteine) proteinase inhibitor, clade A, member 3K
SSC	Sodium chloride/sodium citrate buffer
TAG	Triacylglycerols
TMR	Tetramethylrhodamine
Tris-HCL	Tris (hydroxymethyl)aminomethane/hydrochloric acid
UGT2b5	UDP-glucuronosyltransferase 2 family member 5
V	Voltage
VLACS	Very long-chain acyl-CoA synthetase
VLDL	Very low density lipoprotein
VLFA	Very long-chain fatty acid
W	Watt
wk	Weeks
Wy	Wy-14,643 [4-chloro-6(2,3-xylindino)2-pyrimidinylthiolacetic acid]
Wy-14,643	4-chloro-6(2,3-xylindino)2-pyrimidinylthiolacetic acid
X-Gal	5-bromo-4-chloro-3-indolyl-D-thiogalactoside
8-OH-dG	8-Hydorxydeoxyguanosine

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Chapter 1 Literature review

Peroxsiome proliferator activated receptor (Aldridge *et al.*) was first cloned from mouse liver in 1990 (Issemann and Green, 1990). There are three isoforms: PPARα, PPARδ (also known as NUCI and PPARβ), and PPARγ. Among the three isoforms, PPARα is involved in lipid metabolism (Dreyer *et al.*, 1993), short-term peroxisome proliferator-induced pleiotropic responses (Lee *et al.*, 1995) and long-term hepatocarcinogenesis (Peters *et al.*, 1997). Identification of the genes that regulated by PPARα and affected by peroxisome proliferators can help us to understand more about the mechanism(s) of peroxisome proliferator-induced liver cancer. Monitoring the early expression of peroxisome proliferators-responsive and PPARα-dependent genes can be useful to prevent the induction of cancer by new peroxisome proliferator chemical.

1.1 Peroxisome proliferator activator receptors

Peroxisome proliferator activated receptors (PPARs) are belonging to the large steroid/retinoid nuclear receptor family which has a common modular structure. It consists of six functional domains, defined as A and B (transactivation), C (DNA-binding), and D, E, and F (ligand-binding, dimerization and transactivation) (Motojima, 1993; Mangelsdorf *et al.*, 1995; Kota *et al.*, 2005). The A and B domains

are ligand-independent transcriptional activation domains (activation function-1) (Werman *et al.*, 1997; Kota *et al.*, 2005). They are active in some cell types and responsible for phosphorylation of PPAR. The C domain that is DNA-binding domain (DBD) consisting of two zinc finger DNA binding motifs. The DNA-binding domain promotes the binding of PPAR to the PPRE in the promoter region of target genes. The D domain is the docking region for cofactors. The E domain (LBD) is responsible for ligand specificity and activation of PPAR binding to the PPRE that increases the target gene expressions. Modulators of transcription such as coactivators and corepressors interact with ligand-dependent activation function 2 (AF-2), which located in the E/F domain .

PPARs heterodimerize with the retinoid X receptors (RXRs) and bind to the PPRE of target genes. *In vitro*, when the PPARs and RXRs present at high concentrations, they will heterodimerize, but stimulation by ligands are required at the lower concentrations of the receptors in cells (Forman *et al.*, 1997; Kliewer *et al.*, 1997). The heterodimer binds to a consensus motif named peroxisome proliferator response element (PPRE), which most commonly consisting of the hexad [A/G]GGTCA directly repeated with a one nucleotide spacer (DR-1 elements) (Kliewer *et al.*, 1992). It is reported that PPARs also bind other sites such as DR-2 elements (Everett *et al.*, 2000). PPAR-RXR heterodimers interacting at a PPRE can

respond to either PPs or the RXR ligand, 9-*cis*-retinoic acid. Some retinoids can activate the PPAR-RXR heterodimer through binding RXRs. They exhibit peroxisome proliferation and induction of the enzymes that involved in β-oxidation of fatty acids, which is induced by peroxisome proliferators. PPARs cannot bind to the PPREs of the target genes without interacting with the RXR to form the heterodimer (Gearing *et al.*, 1993; Keller *et al.*, 1993). PPRE is identified in the 5' upstream regions of several target genes and as an almost perfect direct repeat of the sequence TG(A/T)CCT separated by one base pair in the genes of the peroxisomal acyl CoA oxidase (AXO), bi-functional enzyme (PBFE), and thiolase, the microsomal CYP4A subfamily and the cytosolic L-FABP (Lee *et al.*, 1995).

To date, there are three major types of PPAR have been identified: PPARα, PPARβ/δ and PPARγ which perform different physiological functions based on their tissue-specific expression (Table 1.1.1). PPARα is highly expressed in liver, kidney, muscle and fat body in rodents (Kliewer *et al.*, 1994; Braissant and Wahli, 1998). However, the level of PPARα expression in humans is lower than in rodents (Palmer *et al.*, 1998) and higher levels in heart, kidney, skeletal muscle and large intestine kidney and muscle (Mukherjee *et al.*, 1997). PPARα regulates the fatty acid metabolism through stimulating peroxisomal and mitochondrial β-oxidation and microsomal ω-oxidation. PPARα also involves in detoxification of endogenous and

Table 1.1.1. Tissue distribution and physiological role of PPARα, β & γ in rodents

Isoforms	Tissue distribution	Physiological role
α	Liver, kidney, muscle and adipose tissue	Lipid metabolism Regulation of inflammation Detoxification of endogenous and exogenous chemcials
β/δ	Expressed ubiquitously	Embryo implantation Decidualization
γ	Adipose tissue, spleen, intestine	Adipocyte differentiation Regulation of inflammation

exogenous active molecules, some of which may be PPAR ligands (Beck *et al.*, 1992) and down-regulates inflammatory responses (Devchand *et al.*, 1996; Staels *et al.*, 1998). PP-induced pleiotropic responses shown in recombinant adenovirus that expresses human and mouse PPARα indicated that human PPARα is functionally competent and is equally as dose-sensitive as mouse PPARα in inducing peroxisome proliferation (Yu *et al.*, 2001). PPARβ/δ is ubiquitously expressed in both rodents and humans and it is expressed at low level in liver and kidney (Kliewer *et al.*, 1994; Braissant *et al.*, 1996). The physiological function of PPARβ/δ is not yet well defined, previous reports have mentioned that it is involved in embryo implantation and decidualization (Lim *et al.*, 1999). PPARγ exists in two distinct isoforms PPARγ1 and PPARγ2, which PPARγ1 is expressed in liver but expressed in lower levels in adipose tissue and PPARγ2 is expressed in adipose tissue and is a potent regulator of adipocyte differentiation. It is also expressed in marcrophages, fat-storing cells such as hepatic stellate cells, liver, lung, kidney, placenta and colon. The expression of PPARγ in liver is at higher level in human when compared with that in rodent (Tugwood *et al.*, 1996). PPARγ inhibits angiogenesis and inflammatory processes, plays a role in adipocyte differentiation and regulates adipocyte-specific gene expression (Tontonoz *et al.*, 1994; MacDougald and Lane, 1995).

PPARα is qualitatively and quantitatively different between rodent and human.

The levels of PPARα mRNA and DNA binding activity are considerably lower in human liver samples than in rat liver. There is sequence variation of the human PPARα that results in a truncated protein incapable of binding DNA that makes up 10-40% of human PPAR mRNA in liver. It is reported that human liver contains a functionally active PPARα, however, the human PPARα is expressed only about 10% of that in mouse liver, and extracts from human liver contain little PPARα that can bind to a PPRE (Palmer *et al.*, 1998). Sequence variant of PPAR (hPPAR6/29) that varies at three residues from the first cloned sequences is also found. This sequence variant can bind DNA but is not activiated by PPAR ligands, which plays a role as dominant negative suppressor.

1.2 Peroxisome proliferators

Peroxisome proliferators (PPs) are the group of chemicals which stimulate proliferation of peroxisomes. They consist of a broad spectrum of chemicals with little structural similarity, except for the presence of an aromatic ring, carboxylic acid and aliphatic chain. Most peroxisome proliferators are amphipathic molecules containing a hydrophobic backbone (aliphatic or aromatic) linked to an acidic function, which is essential for ligand activity. (Corton *et al.*, 2000).

This group of chemicals (Table 1.2.1) include naturally occurring steroids such

Table 1.2.1. Natural and synthetic PPARα ligands

Natural ligands		
	Saturated fatty acids	
		Palmitic (16:0)
		Stearic (18:0)
	Monounsaturated fatty acid	
		Palmitoleic (16:1)
		Oleic (18:1)
		Elaidic (20:1)
	Polyunsaturated fatty acids	
		Linoleic (18:2, n-6)
		Arachidonic (20:4, n-3)
		Eicosapentaenoic (22:5, n-3)
		Docosahexaenoic (22:6, n-3)
	Eicosanoids	
		Prostaglandins A1
		Leukotriene B4
Synthetic ligands		
	Plasticizers	
		DEHP
		DBP
		DEHA
	Industrial solvent	
		Trichloroethylene
	Herbicides	
		Lactofen
		Fomasafen
		Haloxyfop
	Hypolipidemic drugs	
		Clofibric acid
		Fibrates
		Wy-14,643

as dehydroepiandrosterone (DHEA) (Frenkel *et al.*, 1990), fatty acid such as including palmitic acid, oleic acid, linoleic acid and arachidonic acid (Gottlicher *et al.*, 1992) and ecosanoids (Forman *et al.*, 1997) such as the inflammatory mediator leukotriene B_4 (Devchand *et al.*, 1996).

PP also includes some synthetic compounds (Table 1.2.1) such as di(2-ethylhexyl)phthalate (DEHP) (Okita and Chance, 1984) which has been listed by the International Agency for Research on Cancer (IARC) and by the National Toxicology Program as a possible or reasonably anticipated human carcinogen because it induces dose-related increases in liver tumors in both sexes of rats and mice (IARC, 1982; Melnick, 2001) and di-(2-ethylhexylexyl)adipate (DEHA) (Bell, 1983). Hypolipidemic drugs such as clofibrate (Reddy and Qureshi, 1979), Wy-14,643 [4-chloro-6(2,3-xylidino)-2-pyrimidinyl-thio] acetic acid (Reddy and Krishnakantha, 1975) and nafenopin also have marked hepatomegalic and peroxisome-proliferative properties. Chronic nafenopin treatment developed hepatocellular carcinomas, some of which metastasized to the lungs in acatalasemic mice (Reddy *et al.*, 1976). Other synthetic peroxisome proliferators include herbicides such as lactofen (1'[carboethoxy]ethyl 5-[2-chloro-4-[trifluoro-methyl] phenoxy]-2-nitrobenzoate) which induces liver tumor in mice, pure and technical grade lactofen appear to induce murine liver tumors through a mechanism similar to epigenetic hepatocarcinogens of

the peroxisome proliferating type (Butler *et al.*, 1988), fomesafen that causes peroxisome-related tumours in the mouse, man is neither susceptible nor sensitive to this mechanism (Smith and Elcombe, 1989) and haloxyfop [2-(4-((3-chloro-5-(trifluoromethyl)-2- pyridinyl)oxy)phenoxy)propanoic acid] . Ingestion of haloxyfop by rats and/or mice (0.1-14 mg/kg/day for 2 to 4 weeks) resulted in significant dose-related PP as evidenced by hepatocellular hypertrophy, increased peroxisome volume density (VD) and induction of peroxisomal enzymes and CYP4A1 (Stott *et al.*, 1995). Trichloroethylene (TCE) is an industrial solvent and a widespread environmental contaminant and identified as a weak PP (Zhou and Waxman, 1998; Laughter *et al.*, 2004).

Peroxisome proliferators may activate PPARs by binding directly to the receptor or indirectly to generate PPAR ligands by perturbing lipid metabolism (Corton *et al.*, 2000). Peroxisome proliferators may act indirectly by increasing the level of the free fatty acids as some peroxisome proliferators can bind to liver fatty-binding protein and displace fatty acids such as oleic acid (Green, 1995).

1.2.1 Hepatomegaly

Treatment with PP results in rodents in pleiotropic responses, one of the morphological changes is hepatomegaly. A histological method utilizing the optical

dissector principle indicated that the hepatomegaly induced by the peroxisome proliferator gemfibrozil is a combination effect of hypertrophy and hyperplasia (Carthew *et al.*, 1998). Treatment with clofibrate, nafenopin, tibric acid and Wy-14,643 produced a marked hepatomegaly in the mice. The increase in liver weight correlated well with the increase in total hepatic DNA and in the collective volume of hepatocyte peroxisomes (Moody and Reddy, 1978). Treatment with different peroxisome proliferators, including clofibrate, ciprofibrate, nafenopin, gemfibrozil, Wy-14,643, DEHP and DEHA at carcinogenic doses on male F-344 rats for 1 week shown a close correlation between relative liver weights and hepatocarcinogenicity (r = 0.910). (Takagi *et al.*, 1992). This suggested that the hepatomegaly may be an early biomarker for prediction of the potential hepatocarcinogenicity of peroxisome proliferators. A strong correlation between the animal thyroid status and both hepatocyte proliferation (r2 = 0.62) and hepatocyte surface area (r2 = 0.83) suggested that the thyroid status of the animal was important in PPARα-mediated hepatocellular proliferation and liver cell enlargement (Wang *et al.*, 2004). Treatment with clofibrate and Wy-14,643 did not result in significant hepatomegaly in the PPARα (-/-) mice when compare with the untreated PPARα (+/+) mice (Lee *et al.*, 1995). This indicated that the PP-induced hepatomegaly is mediated by PPARα.

1.2.2 Peroxisome proliferation

Peroxisome proliferation means the increase in both the sizes and the number of peroxisomes and is induced by peroxisome proliferators such as clofibrates and Wy-14,643. High fat diets, starvation and cold termperatures may also stimuate peroxisome proliferation (Gonzalez, 1997). Peroxisome proliferation has been reported to be strongly associated with but not causally to tumour formation (Green, 1995). Di(2-ethylhexyl) adipate (DEHA) is a strong peroxisome proliferator but weakly carcinogenic. It is reported that the peroxisome proliferator such as DEHA may only partially activate peroxisome proliferators-activated receptors (PPARs) while some chemicals may fully activate PPARs. Chemicals, which stimulate only part of the receptor's activity will cause peroxisome proliferation while complete activation is required to stimulate tumourigenesis. Peroxisome proliferation is also proposed as a biomarker of exposure to a variety of pollutants in the environmental pollution assessment (Cajaraville *et al.*, 2003).

It is suggested that the PP-induced peroxisome proliferation was due to the increase in rate of transcription of genes encoding peroxisomal fatty acid β-oxidation enzymes. The increase in level of mRNA of specific genes will lead to increase in expression of specific protein that will accumulate within smooth membrane channels and result in peroxisome proliferation (Dzhekova-Stojkova *et al.*, 2001).

PP-induced peroxisome proliferation is not observed in PPARα (-/-) mice with the administration of classical peroxisome proliferators, clofibrate and Wy-14,643. It indicates that PPARα is the major isoform required for mediating PP-induced peroxisome proliferations (Lee *et al.*, 1995). PPARα-humanized mice shown induction of genes involved in peroxisomal and mitochondrial fatty acid metabolism and resultant decrease of serum triglycerides upon treatment with Wy-14,643. However, hepatocellular proliferation was not observed in PPARα-humanized mice. This suggested that the PPARα-mediated pathways controlling lipid metabolism are independent from those controlling the cell proliferation pathways (Cheung *et al.*, 2004).

1.2.3 Target genes regulation

Induction of enzymes that involved in peroxisomal and mitochondrial β-oxidation and microsomal ω-oxidation of fatty acids is observed after administration of peroxisome proliferators. For peroxisomal β-oxidation, induction of acyl coenzyme A (acyl-CoA) oxidase (AXO), bifunctional enzymes (PBFE) and 3-ketoacylCoA thiolase (thiolase) that located in the peroxisomal matrix are induced with the administration of peroxisome proliferators and they are direct targets of PPARα (Desvergne and Wahli, 1999). The fold of induction of AXO, BIEN and

thiolase is about 10- to 30-fold, whereas catalase and urate oxidase activities are increased 2- to 3-fold (Reddy *et al.*, 1986; Nemali *et al.*, 1988; Nemali *et al.*, 1989).

Soluble epoxide hydrolase (sEPHX) is also induced in rat liver with the peroxisomal β-oxidation by peroxisome proliferators with 5- to 10-fold increase (Grant *et al.*, 1993). Dramatic increases in the activities of other peroxisomal enzymes, such as carnitine acetyltransferase and carnitine octanoyltransferase also occur in the livers. PPARα further regulates the mitochondrial β-oxidation by modulating the expression of medium-chain acyl-CoA dehydrogenase (MCAD) gene (Desvergne and Wahli, 1999). The microsomal p450 4A family (Cyp4a1, 4a2 and 4a3) that involved in the ω-hydroxylation is induced upon the treatment with peroxisome proliferators and with 2- to 5-fold induction (Reddy and Mannaerts, 1994).

PPARα also mediates the expressions of genes that involved in other metabolisms including biotransformation, ketogenesis, fatty acid activation, fatty acid binding and transport, lipoprotein metabolism, lipogenesis, inflammation, triglyceride clearance, gluconeogenesis, phospholipids secretion, cholesterol catabolim and amino acid metabolism (Mandard *et al.*, 2004) (Table 1.2.3.1).

There are sequence variations in PPAR target genes that alter the response to peroxisome proliferators. For example, human acyl-CoA oxidase promoter is

Table 1.2.3.1. List of PPARα regulated genes in liver

Function of gene	Genes	
Amino acid metabolism	Alanine aminotransferase	
	Alanine glyoxylate transaminase	Cytosolic aspartate aminotransferase
	Arginase	Glutaminase
	Arginino-succinate lyase	Hydroxypyruvate/ gloxylate reductase
	Arginino-succinate synthase	Mitochondrial aspartate aminotransferase
	Carbamoyl-phosphate synthase-I	Ornithine transcarbamoylase
Acute phase response	Alpha-1 acid glycoprotein/serine protease inhibitor	Fibrinogen
Bile acid metabolism	Cytochrome P450 7A1	
Bile salt transport	Na+ taurocholate cotransporting polypeptide	
Biotransformation	Aldehyde dehydrogenase type 3	Glutathione-S-transferase alpha
	Cytochrome P450 2A5	Cytochrome P450 3A11
	Cytochrome P450 2C29	UDP-glycuronosyltransferase 1A9
	Cytosolic epoxide hydrolase	
Cell cycle	c-myc	Cyclin D1
Cell cycle regulation	G0/G1 switch gene 2	
Cellular proliferation	glucose-regulated protein-94	
Cholesterol metabolism	Cytochrome P450 12A1	Liver X receptor α
	Cytochrome P450 27A1	
Cholesterol transport	ABCA-a (via LXRα)	ABCG-8 (via LXRα)
	ABCG-5 (via LXRα)	
Desaturation of fatty acid	Delta-6-Desaturase	Stearoyl-CoA desaturase
	Delta-5-desaturase	
Fatty acid activation	Long-chain acyl-CoA synthetase	
Fatty acid binding/transport	Cytosolic fatty acid binding protein	
Fatty acid transport	Fatty acid transport protein-1	Fatty acid translocase
Fatty acid β-oxidation	Acyl-CoA oxidase	Medium-chain acyl-CoA dehydrogenase
	ATP-binding cassette half-transporter type 2	Long-chain acyl-CoA dehydrogenase
	ATP-binding cassette half-transporter type 3	Short-chain-specific 3-ketoacyl-CoA thiolase
	Bifunctional enzyme	Short-chain acyl-CoA
	Carnitine palmitoyl-transferase I	Sterol carrier protein-X
	Carnitine palmitoyl-transferase II	Thiolase B
	Dodecenoyl-CoA delta-isomerase	Very long chain acyl-CoA dehydrogenase
Fatty acid ω-hydroxylation	Cytochrome P450 4A1	Cytochrome P450 4A10
	Cytochrome P450 4A3	Cytochrome P450 4A14
	Cytochrome P450 4A6-Z	
Fatty acid synthesis	Malic enzyme	
Fatty acyl-CoA ester transport	Acyl-CoA binding protein	
Gluconeogenesis	Lactate dehydrogenase A4	

Table 1.2.3.1. List of PPARα regulated genes in liver (con't)

Function of gene	**Genes**	
Glucose oxidation	Pyruvate dehydrogenase kinase isoform 4	
Heme biosynthesis	Coproporphyrinogen oxidase	
Hepatic sterol	Farnesoid X receptor/Bile acid receptor	
Hepatobiluary lipid transport	Multidrug resistance 2	
Hydrolysis of Acyl-CoA	Cytosolic acyl-CoA thioesterase-I	Peroxisomal acyl-CoA thioesterase 1A
	Cytosolic acyl-CoA thioesterase-II	Peroxisomal acyl-CoA thioesterase 1B
	Mitochondrial acyl-CoA thioesterase-I	Peroxisomal acyl-CoA thioesterase 2
Iron-binding protein	Lactoferrin	
Inflammation/ apoptosis	IκBα	
Iron transport	Transferrin	
Ketogenesis	Mitochondrial HMG-CoA synthase	Mitochondrial HMG CoA synthase
Oxygen-free radical metabolism	Superoxide dimutase	
Plasma HDL metabolism	Apolipoprotein-AI	Apolipoprotein-AV
	Apolipoprotein-A I (via rev-erbα)	Apolipoprotein-CIII
	Apolipoprotein-AII	Phospholipid transfer protein
	Apolipoprotein-A IV	Scavenger receptor B-type I
Pheromone carrier	Alpha-2 urinary globulin/MUP-I	
Repressor of gene transcription	Rev-erbα	
ROS production	Uncoupling protein-2	
Triglyceride clearance	Lipoprotein lipase	
Steroid metabolism	11-beta-Hydroxysteroid dehydrogenase I	17-beta-hydroxysteroid dehydrogenase IV
Steroid hydroxylase	Cytochrome P450 2C11	Cytochrome P450 2C12
Vascular inflammation	Interleukin-6	Interleukin-6 receptor
	Interleukin-1 recpetor-type 1	

differed from the rat sequence, which leads to the unresponsive of PPARα in transfection studies. On the other hand, the apoAI promoter in humans is responsive to fibrates whereas it is unresponsive for rat promoter as it is differed at three nucleotides. It is reported that PPARα is active in human liver as the human promoter has been shown to respond to fibrates in transgenic animals. This indicates that the responses to peroxisome proliferators between species differs quantitatively and qualitatively (Everett *et al.*, 2000).

1.2.4 Hypolipidemic effect

Numerous research suggested the causative relationship between dyslipidemia and coronary artery disease (CAD). Hypercholesterolemia (high LDL-cholesterol levels), hypoalphalipoproteinmia (low plasma HDL) and hypertriglyceridemia are considered as the risk factors (alone or combined) for CAD (Gervois *et al.*, 2000). Fibrates are the first efficient and widely used lipid-lowering drugs to be used for the treatment of dyslipidemia. High doses (300-1200 mg/day) of fibrates were suggested to achieve efficacious lipid-lowering activity in human (Berger and Moller, 2002). Clofibrate (ethyl-α-p-chlorophenoxyisobutyrate) is probably the most widely used hypolipidemic drug and believed to be low in toxicity (Havel and Kane, 1973).

PPARα mediates the hypolipidemic action of hypolipidemic drugs (e.g. fibrate

and Wy-14,643) in the treatment of hypertriglyceridemia and hypoalphalipoproteinemia (low plasma HDL). The main effect of the hypolipidemic drugs is to lower the triglyceride and cholesterol levels in the plasma (Issemann and Green, 1990). The hypolipidemic effect of the peroxisome proliferators is the enhancement of catabolism of plasma triglyceride-rich lipoprotein due to a decrease in plasma apolipoprotein C-III through the suppression of the apolipoprotein C-III gene expression (Hertz *et al.*, 1995). Gene expression of the lipoprotein lipase, which is important in triglyceride metabolism, is also induced with the treatment of hypolipidemic drugs (Desvergne and Wahli, 1999; Yamazaki *et al.*, 2002). The induction of expression of lipoprotein lipase and increase accessibility of TG-rich lipoprotein particles for lipolysis due to reduced TG-rich lipoprotein apo C-III content will lead to the induction of lipoprotein lipolysis.

PPARα also upregulates HDL that is inversely correlated with the incidence of CAD. It is suggested that HDL play a role from removing and recycling of cholesterol excess from peripheral tissues to liver. The upregulation of HDL is related to the changes in Apo A-I and Apo A-II. However, the responsive was of Apo A-I and Apo A-II in rodents and human are opposite with the application of peroxisome proliferators. Apo A-I and Apo A-II gene transcription is induced in human while their gene transcription is repressed in rodents (Gervois *et al.*, 2000).

Hepatic and cardiac lipid accumulation is observed in the PPARα (-/-) mice as a result of specific inhibition of mitochondrial fatty acid import.

PPARα, which also mediates the lipid metabolism, upregulates the gene expression of enzymes involved in conversion of fatty acids in acyl-CoA esters, fatty acid entry into mitochondria and peroxisomal and mitochondiral fatty acid catabolism (Gervois *et al.*, 2000). These results in the limitation of hepatic TG synthesis and very low density lipoprotein VLDL production.

1.2.5 Hepatocarcinogenesis

Long term administration (11 months) of Wy-14,643 leads to multiple hepatocellular neoplasms, including adenomas and carcinomas (Reddy and Qureshi, 1979; Peters *et al.*, 1997). The hepatocarcinogenicity of peroxisome proliferators must be nongenotoxic mechanisms as they will not cause DNA damage directly (von Daniken *et al.*, 1981). It is reported that there is correlation between the ability of a PP to bind to and/or activate PPARα and the potency of the PP as an inducer of hepatocarcinogenesis. The strongest inducers of hepatocarcinogenesis, Wy-14,643, binds strongly to PPARα and activates PPARα to high levels. In contrast, bezafibrate is a relatively weak hepatocarcinogen and, binds and activates PPARα weakly (Krey *et al.*, 1997).

The hepatocarcinogenesis induced by PPs is a multistage process consisting of hepatocellular hyperplasia and formation of altered hepatocellular foci, adenoma, and carcinoma. This process is reversible before the formation of carcinoma. The progression of PP-induced foci to hepatocellular adenomas requires continual exposure of PPs. Regression of the lesions (decrease proliferation and increase apoptosis) are observed after PP withdrawal (Marsman and Popp, 1994). After removal of nafenopin for five weeks, there is a 20% reduction in the number of hepatocytes in noninvolved tissue and 85% reduction of cells in foci, adenomas, and carcinomas. The data indicates that continual activation of PPARα is necessary for the growth and maintenance of initiated cells in foci, adenomas and carcinomas in the liver of PP-treated rodents (Peters *et al.*, 1997; Kitamura *et al.*, 1999).

Some studies suggested that peroxisome proliferators act as tumour promoters rather than tumour initiators (Cattley *et al.*, 1989; Cattley and Popp, 1989) and these chemicals act upon spontaneously initiated lesions to produce tumours (Cattley *et al.*, 1991; Kraupp-Grasl *et al.*, 1991; Green, 1995). The carcinogenicity of DEHP and Wy-14,643 are related to sustained DNA replication, which induce tumours by influencing the growth of initiated lesion. DEHA and Wy-14,643 are more effective promoting agents in old rats compared with young rats. Since the number of preneoplastic hepatic foci is greater in the older rats, it is suggested that peroxisome

proliferators promote the growth of these 'spontaneously' initiated cells. Identifying any peroxisome proliferator regulated genes that are relevant to the growth of hepatocytes will provide support for this model.

It is reported that there are species differences in response to peroxisome proliferators. PP-induced pleiotropic responses (hepatic hypertrophy, peroxisome proliferation and hepatocellular carcinoma) occured in rodents are not observed in human and primates. Gemfibrozil and fibrates, hypolipidemic drugs used for many years, however, no evidence of elevated peroxisomes and increased liver cancer have shown in the patients (Peters *et al.*, 1997; Everett *et al.*, 2000).

1.3 Mode of actions

Long term (11 months) administration of peroxisome proliferators will cause liver cancer. However, peroxisome proliferators are not directly mutagenic or genotoxic, the mechanism(s) of peroxisome proliferators induced liver is/are not yet confirmed. Many evidences suggest that hepatocarcinogenesis is a multifactorial process consisting of sustained oxidative stress, inhibition of apoptosis and increased cellular proliferation (alteration of cell cycle control).

1.3.1 Oxidative stress

The increase in peroxisomal fatty acid β-oxidation due to administration of peroxisome proliferators (both short-term and long-term) results in a greater production of hydrogen peroxide. The first enzyme in the peroxisomal β-oxidation, acyl-CoA oxidase, generates H_2O_2 during the first step of β-oxidation. However, peroxisomal catalase, which is responsible for the detoxification of hydrogen peroxide, is increase less than 2- to 3-fold (Nemali *et al.*, 1989). The increase in peroxisomal β-oxidation but no induction in catalase will result in an increase in H_2O_2 levels (oxidation stress). This effect is mediated by PPARα as treatment with anti-oxidants can reduce the incidence of tumor formation in rats, which exposed to peroxisome proliferators chronically (Rao *et al.*, 1984).

Excess H_2O_2 can cause DNA damage through formation of adducts such as 8-hydroxyguanime or strand breaks and possibly tumour initiation. 8-Hydorxydeoxyguanosine (8-OH-dG) is a frequently used marker of DNA damage produced by oxygen radicals. It is observed that PPs increase 8-OH-dG levels in livers of treated rodents, but there is no clear link between the observed increase and carcinogenesis (Sausen *et al.*, 1995).

Hypolipidemic drugs such as fibrates have been used for many years, however, no evidence of elevated increased liver cancer have shown in the patients (Peters *et al.*,

1997; Everett *et al.*, 2000). This may be due to the activity of catalase in rodents is located primarily in the peroxisome, whereas it is mainly in the cytoplasm in humans, which make humans have greater protection against oxidative stress.

1.3.2 Inhibition of apoptosis

Apoptosis is one of the defense mechanism against neoplastic transformation. Inhibition of apoptosis is observed with the administration of peroxisome proliferators. Nafenopin and Wy-14,643 inhibit transforming growth factor-β1-induced apoptosis in vitro (Bayly *et al.*, 1994). This interferes the eliminations of cells with damaged or mutated DNA. The inhibition of apoptosis is mediated by PPARα since inhibition of apoptosis by the PPARα ligand nafeopin can be blocked by a dominant negative form of the receptor (James *et al.*, 1998).

1.3.3 Cell proliferation

PP-induced increase in cell-proliferation is observed within 48 hours of PP treatment. With chronic Wy-14,643 treament, cell proliferation is sustained whereas cell proliferation in the liver returns to control levels with weaker PPs e.g. nafenopin. The early increase in cell replication induced by PPs can increase the frequency of spontaneous mutations, which can be fixed by further rounds of cell division or clonal

expansion (Ashby *et al.*, 1994).

1.3.4 Alterations in cell cycle control

It is suggested that peroxisome proliferators will cause alterations in cell cycle control, which might contribute to underlying mechanism of peroxisome proliferator-induced carcinogenesis including inhibition in apoptosis, increase in DNA synthesis and cell replication and alterations in cell division in liver (Marsman *et al.*, 1988).

1.4 Objectives

In order to study the carcinogenic effect of peroxisome prolifertor, Wy-14,643, a potent peroxisome proliferator and hyperlipidemic drug, was selected for the study. It is reported that the treatment of Wy-14,643 will lead to liver cancer in rodents after 1 year treatment, 11 months treatment was performed to identify the PPARα dependent and peroxisome prolifeator-responsive genes at the tumor stage. As the mechanism(s) of PP-induced hepatpcarcinogenesis is/are still not clear, identification of the genes that are PPARα and Wy-14,643 responsive can help to explain the mechanism(s). In order to identify the PPARα-dependent and Wy-14,643-responsive genes that are at the tumor stage, Wy-14,643 was selected for 11 months treatment of the PPARα (+/+) and PPARα (-/-) mice. Temporal expressions of the

PPARα-dependent and Wy-14,643 responsive genes were also studied.

Fluorescent differential display was used to identify the genes, which are regulated by PPARα and affected by peroxisome proliferators, as its sensitive and ability of comparison of more than two treatment groups. Histology was performed to identify the liver damage caused by the chronic administration of Wy-14,643.

The objects of this project are as follows:

1. To examine the liver damage caused by chronic exposure of Wy-14,643.
2. To identify PPARα-dependent and Wy-14,643-responsive genes at the tumor stage
3. To identify the temporal expression of PPARα-dependent and Wy-14,643-responsive genes.

Chapter 2 Materials and Methods

2.1 Animal tail-genotyping

The genotypes of PPARα (+/+) and PPARα (-/-) mice were confirmed by PCR tail-genotyping before the animals were used for feeding treatment as there was no external phenotypic difference between PPARα (+/+) and PPARα (-/-) mice (Figure 2.1.1). PCR tail-genotyping was performed by using a pair of forward (F1, 5'-GCCTGGCCTTCTAAACAT-3') and reverse (R3, 5'-ACTCGGTCTTCTTGATGA-3') primers which located before the *Pst*I (1330-1347) and after the *Sph*I (1503-1520) sites of exon 8 of PPARα gene, respectively (Figure 2.1.2). The expected size of PCR product amplified from genomic tail DNA of PPARα (+/+) mice was 191 bp. Due to the replacement of a 83 bp genomic fragment by a 1.14 kb neo gene between *Pst*I and *Sph*I sites in exon 8 of PPARα gene, the expected size of PCR product amplified from genomic tail DNA of PPARα (-/-) mice was 1248 bp (Lee *et al.*, 2002).

2.1.1 Materials

Isopropanol was purchased from BDH (England). dNTP mix was purchased from Roche (Germany). EDTA was obtained from Riedel-de Haën (Germany). Proteinase K was purchased from Roche (Germany). DNA markers were from Invitrogen (USA). Ethidium bromide and SDS were obtained from Sigma (USA). Forward and reverse primers were synthesized and purchased from Invitrogen (USA). Agarose, boric acid, sodium chloride and trizma base were purchased from USB (USA). *Taq* polymerase and PCR buffer were from GenSys (UK).

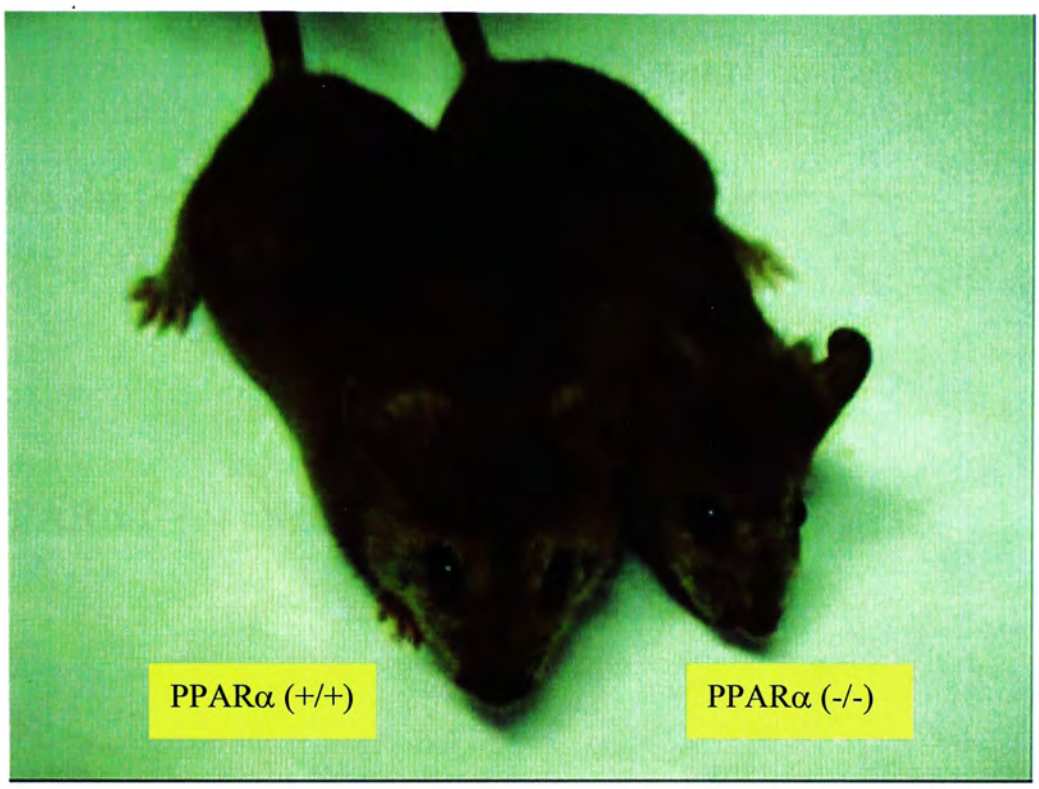

Figure 2.1.1. External morphology of 3 months old PPARα (+/+) and PPARα (-/-) male mice.

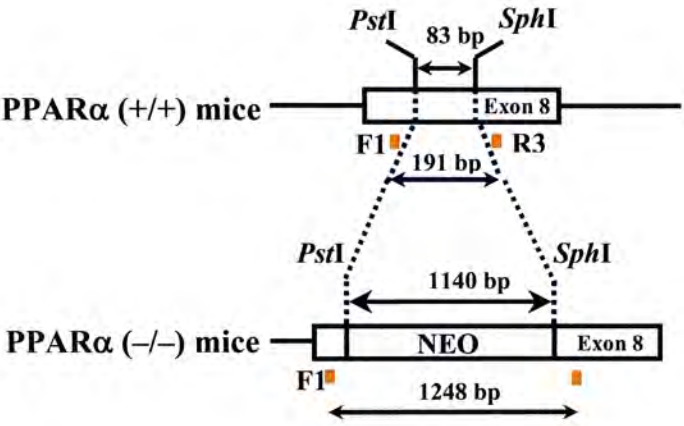

Figure 2.1.2. A schematic representation of PPARα (+/+) and PPARα (-/-) mouse genomic organization and the location of PCR primers designed for tail-genotyping. For PPARα (-/-) mice, a 83 bp genomic fragment between the *Pst*I and *Sph*I sites in exon 8 was replaced by a 1140 bp neo gene. A pair of primers, F1 and R3, which locate before the *Pst*I and after *Sph*I sites, respectively were designed for PCR. The expected sizes of PCR products for PPARα (+/+) mice was 191 bp, while that of PPARα (-/-) was 1248 bp (Lee *et al.*, 2002).

2.1.2 Methods

About 1 cm tail tip from PPARα (+/+) and PPARα (-/-) mice was excised and incubated in 0.5 ml of lysis buffer [100 mM Tris-HCl (pH 8.5), 5 mM EDTA (pH 8.0), 0.2% SDS, 200 mM NaCl and 100 μg/ml proteinase K] in a 15 ml Falcon tube at 55°C with shaking for overnight digestion. After an overnight incubation, the Falcon tube was vortexed and incubated for an additional 2 hr. Then the Falcon tube was centrifuged at 3,000 rpm at room temperature for 10 min and the supernatant was collected into a new 1.5 ml eppendrof tube. The supernatant was centrifuged at 14,000 rpm at room temperature for 10 min and the resulting supernatant was transferred into a new 1.5 ml eppendrof tube which containing 0.5 ml isopropanol. The mixture was shaken laterally to precipitate the DNA and the DNA strand was transferred into a new 1.5 ml eppendrof tube using a P-100 pipette tip. The DNA strand was air-dried, dissolved in 100 μl Tris-EDTA buffer [10 mM Tris-HCl (pH 7.5) and 0.1 mM EDTA (pH 7.5)] and incubated at 55°C water bath overnight. Next day the DNA pellet was resuspended by pipetting up and down and used for PCR reaction.

For each PCR reaction, 1 μl of resuspended tail DNA was mixed with 1X PCR buffer, 0.05 mM dNTP mix, 0.35 μM forward primer (F1), 0.35 μM reverse primer (R3) and 0.1 U/μl *Taq* polymerase (Appendix A, Table A1). The PCR reaction was performed in a thermal cycler (GeneAmp®PCR system 9700) and the PCR cycle was as follows: 95°C for 2 min, followed by 34 cycles of 92°C for 15 sec and 72°C for 2 min and followed by 72°C for 7 min and hold at 4°C. The PCR products of PPARα (+/+) and PPARα (-/-) mice were resolved on 2% and 1% agarose gel, respectively and 0.5X TBE gels with ethidium bromide staining.

2.2 Animal treatment

PPARα (+/+) and PPARα (-/-) mice were used to study the molecular mechanism of PPARα–mediated Wy-14,643 induced hepatocarcinogenesis.

2.2.1 Materials

For all the experiments, PPARα (+/+) and PPARα (-/-) mice on SV129 genetic background were used and they were the offspring of breeder mice obtained from the National Cancer Institute (USA). Wy-14,643 ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]-acetate) was purchased from Chemsyn Science Laboratories (USA) and methylcellulose was obtained from Sigma (USA). Formaldehyde was purchased from Riedel-de Haën (Germany).The mouse chow diet containing 0.0% (control diet) or 0.1% Wy-14,643 was custom-made by Bioserv Company (USA).

2.2.2 Methods

Three months old PPARα (+/+) and PPARα (-/-) male mice were kept in groups of 2-4 mice per cage and provided with ozonated water *ad libitum* on a 12 hr light/dark cycle. For 24 hr gavage treatment, PPARα (+/+) and PPARα (-/-) were given a single dose of 1% methylcellulose (control vehicle) or Wy-14,643 (50 mg/kg bw) in 1% methylcellulose by intragastic infusion. For feeding study, both PPARα (+/+) and PPARα (-/-) mice were either fed with a 0.0% (control) or a 0.1% Wy-14,643 rodent diet for 1 wk, 2 wk, 6 mo and 11 mo *ad libitum*. The body weight of mice was measured before and during (once per week)the feeding periods. At the end of experiment, mice were dissected and blood was collected for serum cholesterol and

triglyceride analyses. Other organs including liver, brown fat, white fat, spleen, kidney, heart, brain and testis were removed and weighed. About 1 cm^3 of liver samples was fixed in 10% phosphate-buffered formalin (10-fold diluted 37% formaldehyde with 1X PBS) for histological analysis. The remaining organs were wrapped in aluminum foil and stored frozen in liquid nitrogen.

2.3 Serum cholesterol and triglyceride analyses

Previous studies have reported that the main effect of the hypolipidemic peroxisome proliferators is to lower the serum triglycerides and cholesterol levels (Havel and Kane, 1973).

Serum cholesterol was determined based on a modification of method described by Allain *et al.* (1974). Cholesterol ester in the serum is first hydrolyzed by cholesterol esterase to cholesterol and fatty acid. The cholesterol is then oxidized by cholesterol oxidase to cholesterone and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide produced is then coupled with 4-aminoantipyrine and phenol to yield quinoneimine, a dye which can be measured at 500 nm. The intensity of the dye is directly proportional to the concentration of serum cholesterol.

Serum triglyceride was determined based on a modification of method described by Bucolo and David (1973). Serum triglyceride is first hydrolyzed by lipase to glycerol and fatty acids. The glycerol is then phosphorylated by adenosine triphosphate (ATP) to glycerol-3-phosphate and adenosine-5'-diphosphate (GDP) which catalyzed by glycerokinase. The glycerol-3-phosphate is then oxidized by glycerol-3-phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide produced is then coupled with 4-

aminoantipyrine and 4-chlorophenol to form quinoneimine which can be measured at 500 nm. The intensity of the colour is directly proportional to the serum triglyceride concentration in the sample.

2.3.1 Materials

Cholesterol and triglyceride determination enzyme kits were obtained from BioSystems (Spain). Plastic curvets were purchased from Nümbrecht (Germany).

2.3.2 Methods

2.3.2.1 Serum preparation

Blood samples were allowed to clot for 30 min at room temperature. The clotted blood samples were then centrifuged at 2,400 rpm at room temperature for 20 min. The supernatant which containing the serum was then transferred to a new 1.5 ml eppendrof tube and centrifuged at 2,400 rpm for 20 min. The clear serum was then transferred to a new 1.5 ml eppendrof tube and stored at -20°C.

2.3.2.2 Serum cholesterol analysis

Cholesterol reagent and cholesterol standard (200 mg/dl) were warmed to room temperature before use. Four microliters of cholesterol standard or serum samples from each mouse were added to curvets containing 400 μl of cholesterol reagent. The contents were mixed and the samples were incubated for 15 min at room temperature. The absorbances (A) of standard and samples at 500 nm were measured using a spectrophotometer (Beckman DU® 640, USA) against the blank which

containing 400 µl of cholesterol reagent. The concentration of serum cholesterol (mg/dl) was calculated using the following formula:

Conc. of serum cholesterol (mg/dl)= A_{sample} / $A_{Standard}$ X conc. of cholesterol standard

2.3.2.3 Serum triglyceride analysis

Triglyceride reagent and triglyceride standard (200 mg/dl) were warmed to room temperature. Four microliters of triglyceride standard or serum samples from each mouse were added to curvets containing 400 µl of triglyceride reagent. The contents were mixed and the samples were incubated for 15 min at room temperature. The absorbances (A) of standard and samples at 500 nm were measured using a spectrophotometer (Beckman DU® 640, USA) against the blank which containing 400 µl of triglyceride reagent. The concentration of serum triglyceride was calculated using the following equation:

Conc. of serum triglyceride (mg/dl)= A_{sample} / $A_{Standard}$ X conc. of triglyceride standard

2.4 Histological analysis

To assess the liver damage induced by Wy-14,643, hematoxylin and eosin staining was performed from each treatment group at 24 hr, 1 wk, 2 wk, 6 mo and 11 mo.

2.4.1 Materials

Mayer's hematoxylin was from Polysciences (USA). Concentrated HCl, ethanol and eosin were purchased from BDH (England). Paraffin was obtained from Merck (USA). Permount was purchased from Fisher (USA). Xylene was from Merck

(USA). Magnesium sulfate and sodium hydrogen carbonate were purchased from BDH (England).

2.4.2 Methods

The flow chart showing the steps for histological analysis is summarized in Figure. 2.4.1. The fixed liver samples were processed in an enclosed tissue processor (Shandon, England) with the following program: 70% ethanol at room temperature for 2 hr, 80% ethanol at room temperature for 2 hr, 95% ethanol at room temperature for 2 hr, 100% ethanol at room temperature for 2 hr three times, xylene at room temperature for 1 hr twice and paraffin at 60°C for 2 hr four times. The processed samples were then embedded in paraffin. Liver sections of 5 μm thick were cut by microtome (Jung, Germany) and then dried at 40°C. For staining, the liver sections were first rinsed with xylene for 1 min three times and then in a serial graded ethanol (100%, 95%, 80% and 70%) for 1 min. The liver sections were stained with Mayer's hematoxylin for 5 min and washed with tap water. The sections were then rinsed with Scott tap water (0.04 M sodium hydrogen carbonate and 0.15 M magnesium sulfate) and washed with tap water. Excess staining was washed by dipping the section in acid alcohol (100-fold diluted concentrated HCl with 70% alcohol) for 1 sec and then washed with tap water. The sections were stained with eosin for 5 min. Excess staining was removed with tap water and the sections were rinsed with a serial graded ethanol (70%, 80% and 95%) for 1 min. The sections were then rinsed with absolute ethanol for 1 min and then followed by rinsing with xylene for 1 min three times. The sections were mounted with permount and photographs were taken with Integrated Biological Imaging System (Axiophot 2 unviersal microscope: Zeiss, Germany; spot

cooled colour digital camera and software-Spot 32: Diagnostic Instruments, USA; Software-MetaMorph 4.0: Universal Imaging Corporation, USA) (Figure 2.4.1).

2.5 Total RNA isolation

Total liver RNA of PPARα (+/+) and PPARα (-/-) mice treated with a 0.0% (control diet) or a 0.1% Wy-14,643 for 11 mo was isolated for non-fluoroDD, fluoroDD and Northern blot analyses. Liver RNA samples of 24 hr gavage feeding and 1 wk, 2 wk and 6 mo feeding treatments were used to study the temporal gene expression.

2.5.1 Materials

Chloroform was from Merck (USA). Ethanol and isopropanol were purchased from BDH (England). Trizol reagent was obtained from Invitrogen (USA).

2.5.2 Methods

The steps for isolation total RNA are summarized in Figure 2.5.2.1. Frozen liver samples were mixed with Trizol reagent (10 ml/g) and homogenized with a tissue tearor (Dremel®, USA). The homogenate was allowed to stand for 5 min and then 1 ml of homogenate was added to a new 2 ml eppendrof tube which containing 0.2 ml chloroform. After vortexing, the mixture was allowed to stand for 2 min and then centrifuged at 14,000 rpm at 4°C for 15 min. The supernatant which containing the RNA was transferred into a new 1.5 ml eppendrof tube which containing 500 μl of isopropanol. The sample was vortexed and then allowed to stand for 10 min at room

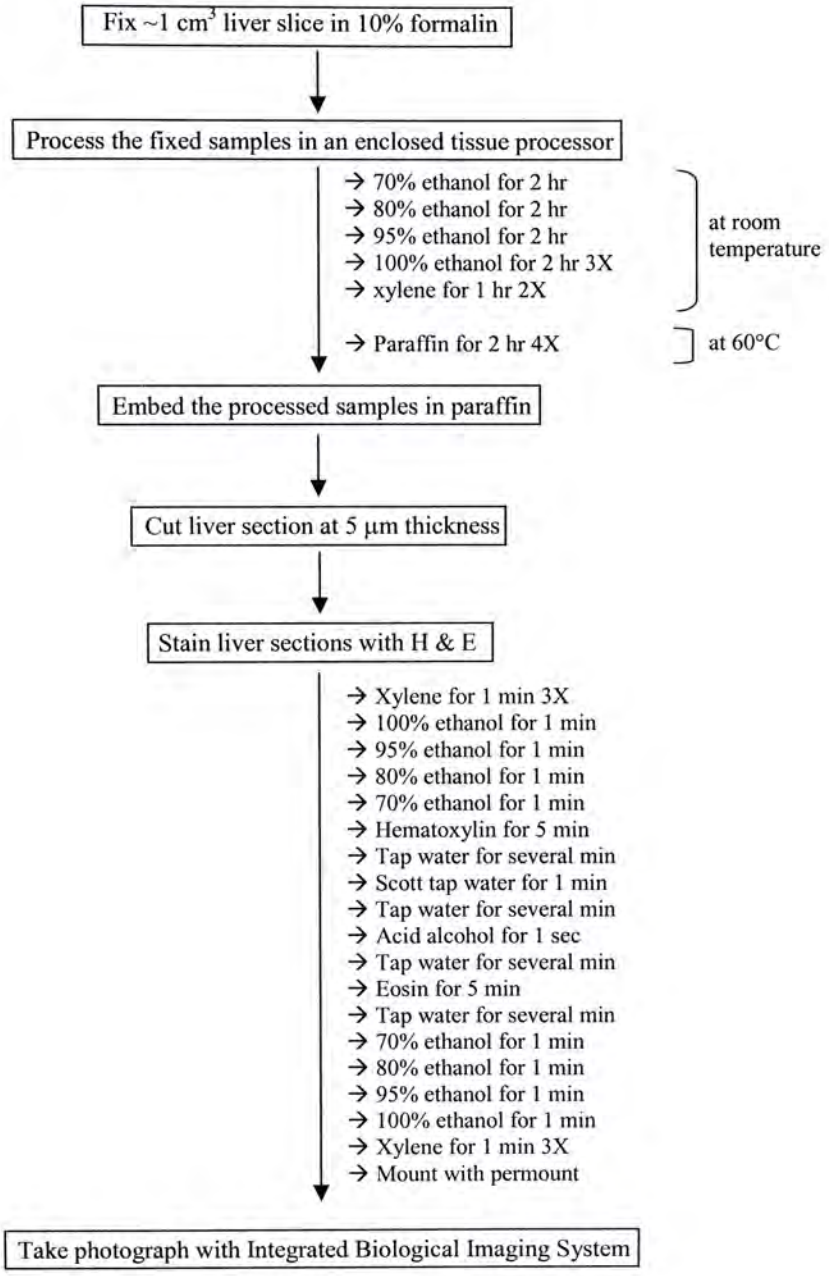

Figure 2.4.1. Flowchart showing the steps for histological analysis.

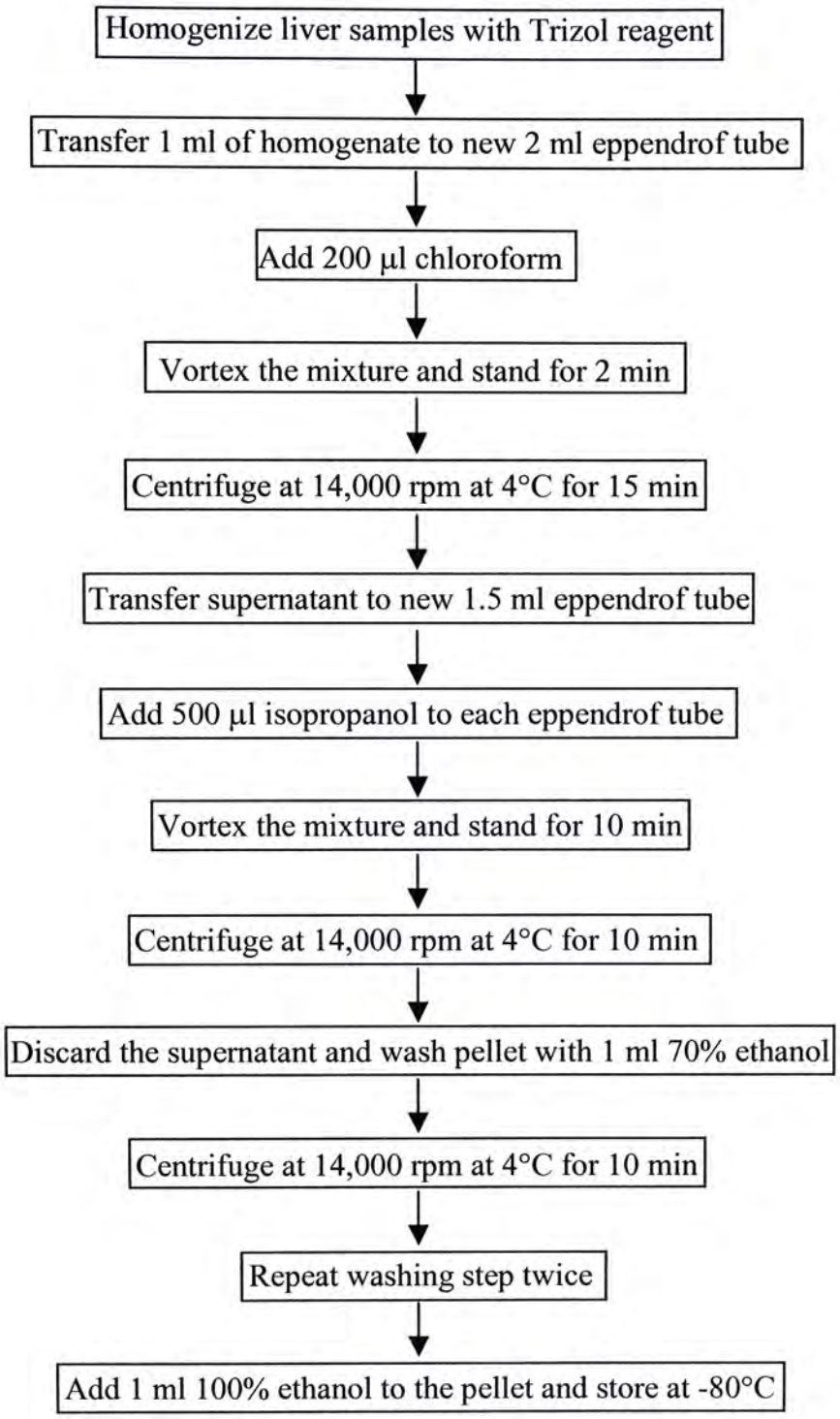

Figure 2.5.2.1 Flowchart showing the steps for isolation of total RNA.

temperature. The mixture was centrifuged at 14,000 rpm at 4°C for 10 min. After discarding the supernatant, the pellet was washed with 1 ml 70% ethanol. The sample was centrifuged at 14,000 rpm at 4°C for 10 min and the supernatant was discarded. The washing steps were repeated twice. For long-term storage, 1 ml of absolute ethanol was added to the pellet and the sample was stored at -80°C.

2.6 DNase I treatment of total liver RNA

DNase I treatment was used to remove any genomic DNA contamination in the RNA samples, as they will give false positive fluoroDD patterns during RT-PCR. Negative control was performed for checking any genomic DNA contamination after DNase I treatment.

2.6.1 Materials

Magnesium chloride and sodium acetate were obtained from Sigma (USA). Nuclease-free water was purchased from Promega (USA). Phenol: chloroform: isoamyl alcohol (25:24:1) was purchased from Invitrogen (USA). RNase-free DNase I, HPLC pure grade was from Amersham Biosciences (England). Trizma base was purchased from USB (USA).

2.6.2 Methods

Fifty micrograms of total liver RNA were mixed with 10 mM $MgCl_2$, 0.3 U/μl RNase-free DNase I and 50 mM Tris-HCl (pH 7.5) (AppendixA, Table A2). The mixture was then mixed by finger-tapping and incubated in 37°C water bath for 30 min. The sample was then diluted to 500 μl with nuclease-free water and 500 μl of

phenol/chloroform was added to each sample. After vortexing and centrifuged at 14,000 rpm at 4°C for 30 min, 400 μl of supernatant was transferred to a new 1.5 ml eppendrof tube. Forty microliters of 3 M sodium acetate were added to each eppendrof tube and vortexed. Eight hundred microliters of absolute ethanol were added to each sample. The mixture was vortexed and allowed for overnight precipitation at -80°C. The precipitated RNA was centrifuged at 14,000 rpm at 4°C for 30 min. The supernatant was discarded and the pellet was washed with 70% ethanol and centrifuged at 14,000 rpm at 4°C for 30 mine. The washing steps were repeated twice and the pellet was air-dried. The RNA pellet was resuspended in 35 μl nuclease-free water and stored at -80°C.

2.7 Reverse transcription (RT) of mRNA and non-fluorescent PCR (non-fluoroDD PCR)

The first-strand cDNA synthesis was carried out by using the HIEROGLYPH^{TM} profile kit. This kit consists of 12 different 3'-oligo(dT) anchored primers (APs) (Figure 2.7.1) which are two-base different on the 3' end and it divides the mRNA pool into 12 first-strand cDNA pools. The double-strand DNA was performed by the fluoroDD adapter kit. It contains 20 ten-base different 5'-arbitrary primers (ARPs) (Figure 2.7.2) and 12 different 3'-tetramethylrhodamine-labeled oligo(dT) anchored primers (TMR-APs) (Figure 2.7.3) which have same sequence to their corresponding anchored primers (APs) in the HIEROGLYPH^{TM} profile kit. There are total 240 different combinations of primers with 12 different APs and 20 different ARPs. However, not all the 240 primer combinations will give our desired differential display patterns, non-fluorescent PCR (non-fluoroDD PCR) (Figure 2.7.4)

T7 promotor sequence — oligo(dT_{12})

AP1	5' ACGACTCACTATAGGGCTTTTTTTTTTTTGA 3'
AP2	5' ACGACTCACTATAGGGCTTTTTTTTTTTTGC 3'
AP3	5' ACGACTCACTATAGGGCTTTTTTTTTTTTGG 3'
AP4	5' ACGACTCACTATAGGGCTTTTTTTTTTTTGT 3'
AP5	5' ACGACTCACTATAGGGCTTTTTTTTTTTTCA 3'
AP6	5' ACGACTCACTATAGGGCTTTTTTTTTTTTCC 3'
AP7	5' ACGACTCACTATAGGGCTTTTTTTTTTTTCG 3'
AP8	5' ACGACTCACTATAGGGCTTTTTTTTTTTTAA 3'
AP9	5' ACGACTCACTATAGGGCTTTTTTTTTTTTAC 3'
AP10	5' ACGACTCACTATAGGGCTTTTTTTTTTTTAG 3'
AP11	5' ACGACTCACTATAGGGCTTTTTTTTTTTTAT 3'
AP12	5' ACGACTCACTATAGGGCTTTTTTTTTTTTCT 3'

Figure 2.7.1 Sequences of 12 different 3'-oligo(dT_{12}) anchored primers (APs). Each AP primer contains three domains and they are the same for the T7 (17-mer) promoter sequence for amplification and the oligo(dT_{12}) for annealing to polyA tail of mRNA. However, the 12 different APs are two-base different at the 3'end which divide mRNA pool into 12 subsets (adapted from fluoroDD manual, Beckman).

	M13 reverse primer sequence Core annealing sequence
ARP1	5' ACAATTTCACACAGGACGACTCCAAG 3'
ARP2	5' ACAATTTCACACAGGAGCTAGCATGG 3'
ARP3	5' ACAATTTCACACAGGAGACCATTGCA 3'
ARP4	5' ACAATTTCACACAGGAGCTAGCAGAC 3'
ARP5	5' ACAATTTCACACAGGAATGGTAGTCT 3'
ARP6	5' ACAATTTCACACAGGATACAACGAGG 3'
ARP7	5' ACAATTTCACACAGGATGGATTGGTC 3'
ARP8	5' ACAATTTCACACAGGATGGTAAAGGG 3'
ARP9	5' ACAATTTCACACAGGATAAGACTAGC 3'
ARP10	5' ACAATTTCACACAGGAGATCTCAGAC 3'
ARP11	5' ACAATTTCACACAGGAACGCTAGTGT 3'
ARP12	5' ACAATTTCACACAGGAGGTACTAAGG 3'
ARP13	5' ACAATTTCACACAGGAGTTGCACCAT 3'
ARP14	5' ACAATTTCACACAGGATCCATGACTC 3'
ARP15	5' ACAATTTCACACAGGACTTTCTACCC 3'
ARP16	5' ACAATTTCACACAGGATCGGTCATAG 3'
ARP17	5' ACAATTTCACACAGGACTGCTAGGTA 3'
ARP18	5' ACAATTTCACACAGGATGATGCTACC 3'
ARP19	5' ACAATTTCACACAGGATTTTGGCTCC 3'
ARP20	5' ACAATTTCACACAGGATCGATACAGG 3'

Figure 2.7.2 Sequences of 20 different 5'-arbitrary primers (ARPs). Each ARP primer contains the same M13 (16-mer) reverse primer sequence for reamplification but they have ten-base different for the core annealing sequence for priming gene specific sequence in RT-PCR (adapted from fluoroDD manual, Beckman).

T7 promotor sequence oligo(dT_{12})

TMR-AP1	★5' ACGACTCACTATAGGGCTTTTTTTTTTTTGA 3'
TMR-AP2	★5' ACGACTCACTATAGGGCTTTTTTTTTTTTGC 3'
TMR-AP3	★5' ACGACTCACTATAGGGCTTTTTTTTTTTTGG 3'
TMR-AP4	★5' ACGACTCACTATAGGGCTTTTTTTTTTTTGT 3'
TMR-AP5	★5' ACGACTCACTATAGGGCTTTTTTTTTTTTCA 3'
TMR-AP6	★5' ACGACTCACTATAGGGCTTTTTTTTTTTTCC 3'
TMR-AP7	★5' ACGACTCACTATAGGGCTTTTTTTTTTTTCG 3'
TMR-AP8	★5' ACGACTCACTATAGGGCTTTTTTTTTTTTAA 3'
TMR-AP9	★5' ACGACTCACTATAGGGCTTTTTTTTTTTTAC 3'
TMR-AP10	★5' ACGACTCACTATAGGGCTTTTTTTTTTTTAG 3'
TMR-AP11	★5' ACGACTCACTATAGGGCTTTTTTTTTTTTAT 3'
TMR-AP12	★5' ACGACTCACTATAGGGCTTTTTTTTTTTTCT 3'

Figure 2.7.3 Sequences of 12 different 3'-tetramethylrhodamine-labeled oligo(dT_{12}) anchored primers (TMR-APs). Each TMR-AP primer has same sequence corresponding to the non-fluorescent-labeled anchored primer used for first-strand cDNA synthesis and the TMR fluorescent tag is indicated by the star (adapted from fluoroDD manual, Beckman).

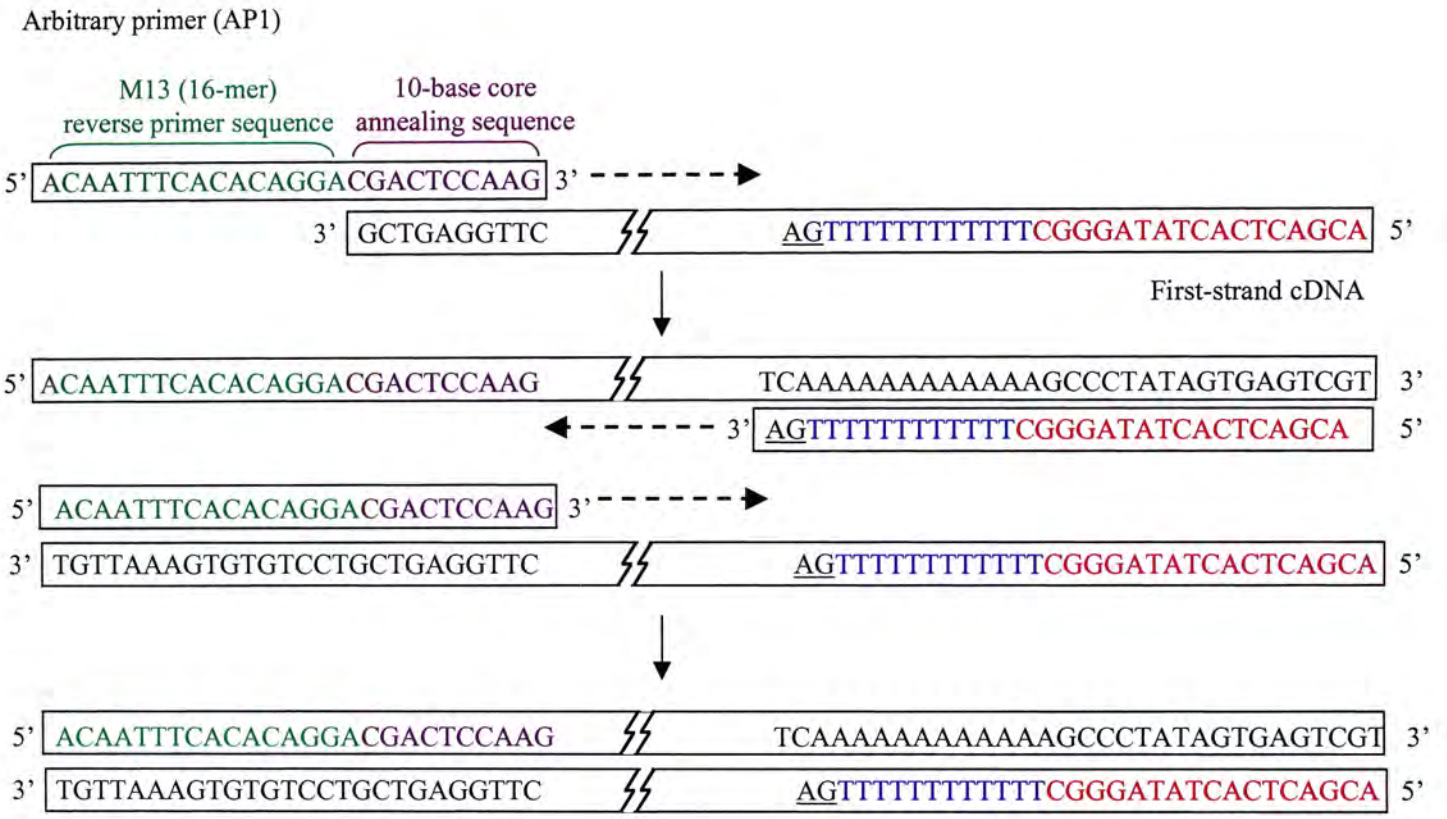

Figure 2.7.4. A schematic diagram showing the synthesis of non-fluoroDD PCR. Anchored primer (AP) and arbitrary primer (ARP) are used for the double-strand cDNA synthesis (adapted from fluoroDD manual, Genoymx).

was performed for initial screening of primer combinations which produced our desired differential display patterns. Non-fluorescent labeled APs were used in the non-fluoroDD RT-PCR which provides a cheaper and faster screening method.

2.7.1 Materials

DTT, Superscript™ II RT buffer and Superscript™ II RNase H⁻ reverse transcriptase, non-fluorescent labeled 3'-oligo(dT) anchored primers (APs), 5'-arbitrary primers (ARPs), dNTP mix (1:1:1:1), *Taq* polymerase and PCR buffer were obtained from Invitrogen (USA).

2.7.2 Methods

A schematic diagram showing the synthesis of non-fluoroDD PCR is shown in Figure 4.7.5. First-strand cDNA was synthesized from DNase I treated total RNA (0.2 μg) by mixing with 0.2 μM AP, 10 mM DTT, 25 μM dNTP mix (1:1:1:1), 1X Superscript™ II RT buffer and 2 U/μl Superscript™ II RNase H⁻ reverse transcriptase (Appendix A, Table A3). For negative control, Superscript™ II RNase H⁻ reverse transcriptase was not added for the first-strand cDNA synthesis. The reverse transcription reaction was performed in a thermal cycler (GeneAmp®PCR system 9700) and the RT program was as follows: 42°C for 5 min, 50°C for 50 min, 70°C for 15 min and hold at 4°C. The synthesized first-strand cDNA was stored at -20°C.

After first-strand cDNA synthesis, 4 μl RT mixture was used for the non-fluorescent RT-PCR (non-fluoroDD PCR). For each PCR reaction, the RT mixture was mixed with 0.35 μM AP, 0.35 μM ARP, 50 μM dNTP mix (1:1:1:1), 1X PCR buffer and 0.05 U/μl of *Taq* polymerase (Appendix A, Table A4). The PCR was

performed in a thermal cycler (GeneAmp®PCR system 9700) and the PCR program was as follows: 95°C for 2 min, followed by 4 cycles of 92°C for 15 sec, 50°C for 30 sec, 72°C for 2 min and followed by 30 cycles of 92°C for 15 sec, 60°C for 30 sec, 72°C for 2 min and 72°C for 7 min and hold at 4°C. The double-strand cDNA synthesized was resolved on 1.5% agarose, 0.5X TBE gels with ethidium bromide staining.

2.8 Reverse transcription (RT) of mRNA and fluorescent PCR (fluoroDD PCR)

The AP-ARP primer combinations which showed differential display patterns in non-fluoro RT-PCR were chosen for fluorescent PCR (fluoroDD PCR).

2.8.1 Materials

HIEROGLYPH™ mRNA profile and fluoroDD adpater kits were purchased from Beckman (USA).

2.8.2 Methods

FluoroDD RT-PCR was the same as non-fluoroDD RT-PCR described in sections 4.7.1 and 4.7.2 except that APs and dNTP mix (1:1:1:1) for first-strand cDNA synthesis were purchased from HIEROGLYPH™ mRNA profile kit, while TMR-APs, ARPs and dNTP mix (1:1:1:1) for fluoroDD PCR were obtained from fluoroDD kit. The TMR-labeled cDNA fragments were stored at -20°C.

2.9 Fluorescent differential display (fluoroDD)

The TMR-labeled cDNA fragments were electrophoretically separated on high resolution polyacrylamide gel and detected by fluorescent scanning using GenomyxSC™ fluorescent imaging system.

2.9.1 Materials

FluoroDD loading dye, HR-1000™ 5.6% denaturing polyacrylamide gel, 0.5X TBE buffer, 1X TBE buffer and TMR-labeled molecular weight DNA marker were obtained from Beckman (USA). Ammonium persulfate and TEMED were purchased from Sigma (USA). GenomyxLR™ electrophoresis system and genomyxSC™ fluorescent imaging system were from Genomyx (USA).

2.9.2 Methods

2.9.2.1 FluoroDD gel preparation

High resolution polycarylamide gel was prepared by mixing 70 ml of 5.6% denaturing HR-1000 gel with 320 μl of freshly prepared 10% ammonium persulfate and 32 μl TEMED. The fluoroDD gel was allowed to polymerize for overnight at room temperature.

2.9.2.2 Sample preparation and electrophoresis

Four microliters of fluoroDD PCR products were mixed with 2 μl fluoroDD loading dye, while 1.2 μl TMR-molecular weight marker was mixed with 1.5 μl fluoroDD loading dye. They were denatured at 95°C for 4 min in a thermal cycler (GeneAmp®PCR system 9700) before loading. The lower and upper buffer chambers

were filled with 250 ml 1X TBE buffer and 125 ml 0.5X TBE buffer, respectively. The TMR-molecular weight marker and fluoroDD PCR samples were loaded and run at 3000 V, 100 W at 50°C for 4 to 4.5 hr using a GenomyxLR™ DNA electrophoresis system (Genomyx, USA). The gel was then washed with double distilled water 3 times and dried at 55°C for 20 min. The image was scanned in GenomyxSC™ fluorescent imaging scanner (Genomyx, USA).

2.10 Excision of differentially expressed cDNA fragments

The cDNA fragments which showed differential expression patterns in the Wy-14,643 treated PPARα (+/+) group but not in the Wy-14,643 treated PPARα (-/-) group compared with their corresponding control groups were excised from the fluoroDD gels (Table 2.10.1).

2.10.1 Materials

A virtual grid was purchased from Genomyx (USA). Sterile blades were purchased from Swann-Morton® (England).

2.10.2 Methods

The computer-scanned image of the fluoroDD gel was aligned with a physical grid to accurately locate the positions of cDNA fragments which showed differential display patterns on the fluoroDD gel before gel excision. The fragments which showed differential display patterns were marked on the virtual grid. The cDNA fragments were excised by putting the fluoroDD gel on the virtual grid and excised

Table 2.10.1. The expected differential expression patterns of cDNA fragments displayed on non-fluoroDD and fluoroDD gels.

	PPARα (+/+)		PPARα (-/-)		Description
DD Patttens	**Control**	**0.1% Wy-14,643**	**Control**	**0.1% Wy-14,643**	
I	-	+	-	-	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
II	+	++	+	+	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
III	+	-	+	+	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
IV	++	+	++	++	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
A	-	+	-	+	Up-regulated in 0.1% Wy-14,643 treatment but PPARα-independent
B	+	-	+	-	Down-regulated in 0.1% Wy-14,643 treatment but PPARα-independent

'+'represents the presence of a band (cDNA) on fluoroDD gel and the relative intensity of the gene expression among the four treatment groups is indicated by the number of '+', while '+' represents the corresponding band (cDNA) was not observed on the fluoroDD gel. Those AP and ARP combinations which shown the desired differential display pattern (I-IV) in the non-fluoroDD gels were selected for band excision. Differential display patterns A & B are 0.1% Wy-14,643 treatment responsive but they are not PPARα dependent, so they are not chosen for fluoroDD RT-PCR and gel band excision.

with sterile blades. The excised gel bands were incubated in 20 μl TE buffer [10 mM Tris-HCl (pH 7.5) and 0.1 mM EDTA (pH 7.5)] at 37°C for 1 hr for DNA elution and stored at -20°C. After gel excision, the gels were re-scanned to confirm the target bands were excised accurately.

2.11 Reamplification of differentially expressed cDNA fragments

The excised cDNA fragments were reamplified by PCR reaction with full length M13 reverse (-48) 24-mer (5'-AGCGGATAACAATTTCACACAGGA-3') and T7 promoter 22-mer (5'-GTAATACGACTCACTATAGGGC-3') primers. The partial sequences of M13 reverse (16-mer) and T7 promoter (17-mer) were present in the 5'-ARP and 3'-AP primers of double-strand cDNA synthesis, respectively. As the M13 reverse primer was lengthened by 8 nucleotides and the T7 promoter primer was lengthened by 5 nucleotides, the expected size of reamplified PCR product is 13 nucleotides longer than the size of fluoroDD fragment size (Figure 2.11.1).

2.11.1 Materials

Agarose and trizma base were from USB (USA). dNTP mix (1:1:1:1), *Taq* polymerase and PCR buffer were obtained from Invitrogen (USA). Full-length T7 promoter 22-mer and M13 reverse (-48) 24-mer primers were purchased from Beckman (USA). EDTA and ethidium bromide were purchased from Sigma (USA).

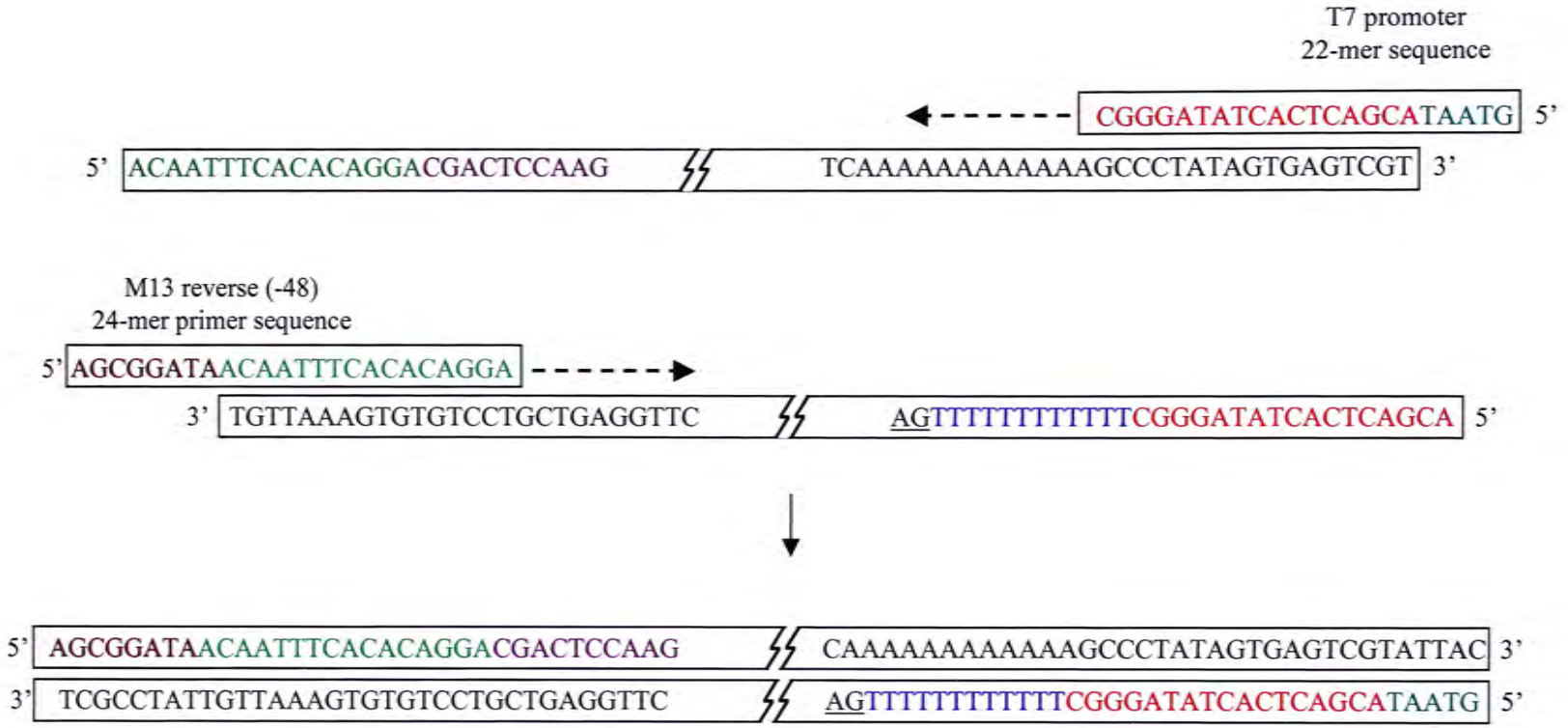

Figure 2.11.1 A schematic diagram showing the reamplification of excised cDNA fragments. Full-length M13 reverse (-48) 24-mer and T7 promoter 22-mer primers wer used for the reamplification of excised cDNA fragments (adapted from fluoroDD manual, Genoymx).

2.11.2 Methods

Four microliters of gel band elute were reamplified by mixing with 20 μM dNTP mix (1:1:1:1), 0.2 μM M13 reverse (-48) 24-mer primer, 0.2 μM T7 promoter 22-mer primer, 1X PCR buffer and 0.05 U/μl of *Taq* polymerase (Appendix A, Table A5). The PCR reaction was performed in a thermal cycler (GeneAmp®PCR system 9700) and the program was as follows: 95°C for 2 min, followed by 4 cycles of 92°C for 30 sec, 50°C for 30 sec, 72°C for 2 min, followed by 25 cycles of 92°C for 30 sec, 60°C for 30 sec, 72°C for 2 min and 72°C for 7 min and hold at 4°C. Two microliters of reamplified PCR product were resolved on 1% or 2% agarose, 0.5X TBE with ethidium bromide staining.

2.12 Subcloning of reamplified cDNA fragments

Subcloning of reamplified cDNA fragments was performed by either AdvaTAge™ PCR or TOPO TA cloning kits. The reamplified cDNA fragments with 3'-A overhang were subcloned by T/A cloning using either pT-Adv or pCR®II-TOPO® vector derived from AdvanTAge™ PCR or TOPO TA cloning kit, respectively. The screening of recombinant subclones was preformed by using phenol-chloroform extraction method (Ohyama, 1997) in which the recombinant plasmid DNA should be longer than the vector only [size of pT-Adv is 3.9 kb (Figure 2.12.1) and that of pCR®II-TOPO® is 4.0 kb (Figure 2.12.2)]. The size of the insert subcloned into the vector was further confirmed with *Eco*RI digestion.

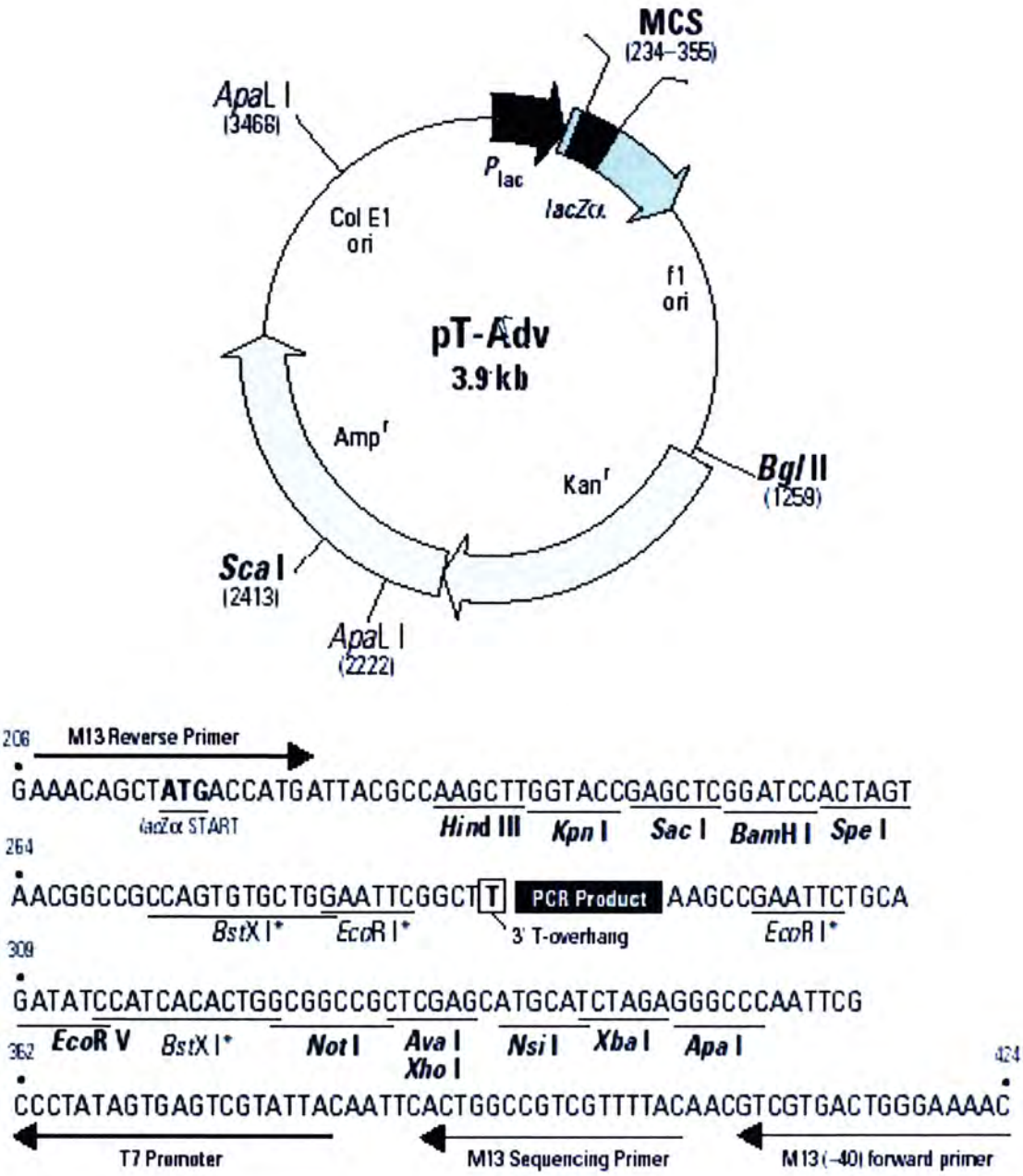

Figure 2.12.1 The restriction map and multiple cloning site (MCS) of pT-Adv vector used for subcloning of reamplified cDNA fragments (adapted from AdvanTAge^{TM} PCR cloning kit user manual).

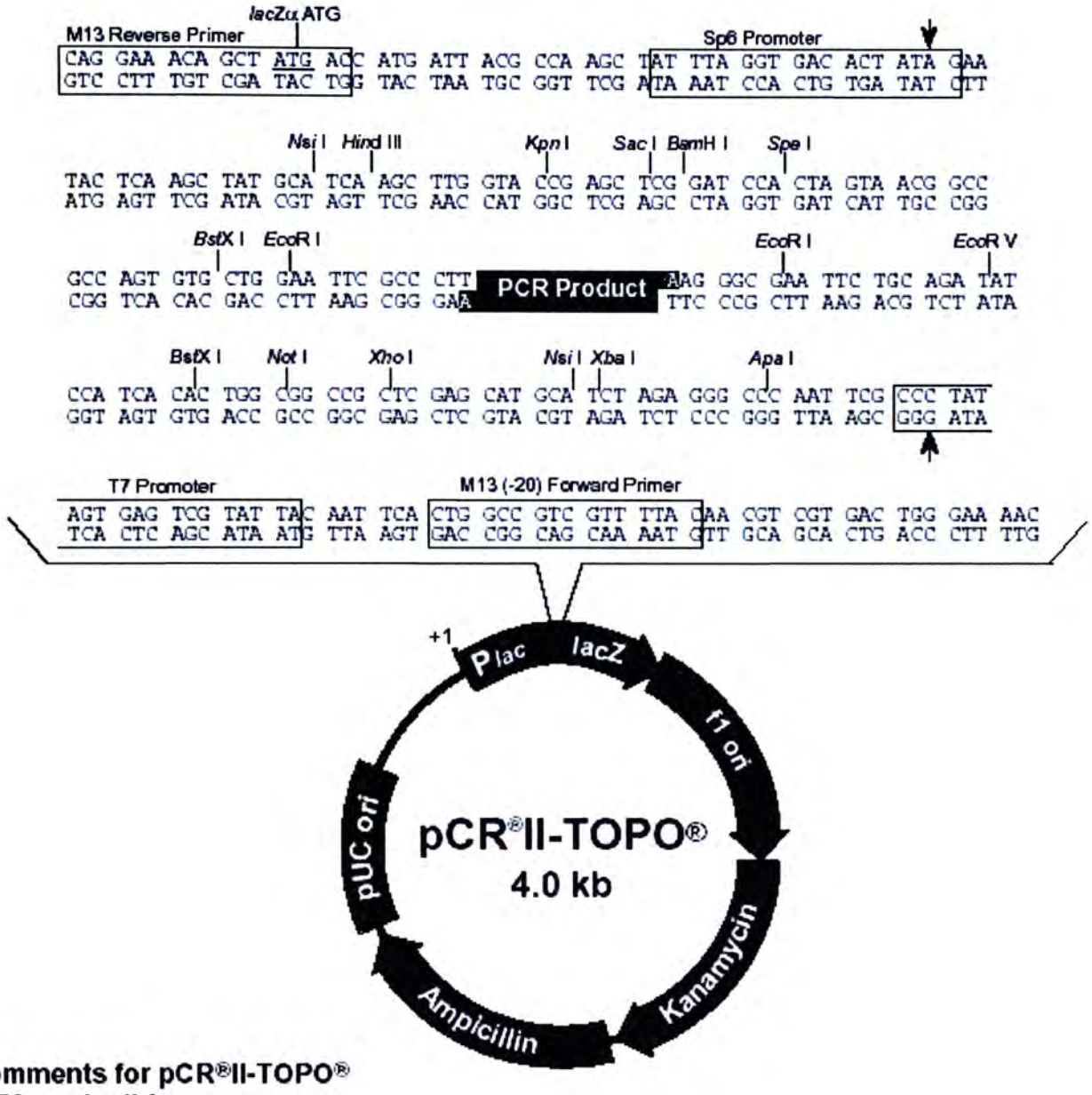

Figure 2.12.2 The restriction map and multiple cloning site (MCS) of PCR® II-TOPO® vector used for subcloning of reamplified cDNA fragments (adapted from TOPO cloning kit user manual).

2.12.1 Materials

Agarose, 5-bromo-4-chloro-3-indolyl-D-thiogalactoside (X-gal), Isopropyl-β-D-thiogalactoside (IPTG) and sodium chloride were obtained from USB (USA). AdvanTAge™ PCR cloning kit was purchased from Clontech (USA). TOPO TA cloning kit was from Invitrogen (USA). Ethidium bromide and ampicillin were purchased from Sigma (USA). Restriction enzymes and buffers were obtained from New England Biolabs (UK). Trypton peptone, yeast extract and bacto agar were purchased from Becton Dickinson (USA). QIAGEN® spin miniprep kit was purchased from QIAGEN (Germany).

2.12.2 Methods

2.12.2.1 Ligation

Subcloning of reamplified cDNA fragments was performed either by AdvaTAge™ PCR or TOPO TA cloning kits. For AdvaTAge™ PCR subcloning kit, 1 μl of reamplified PCR product was mixed with 1X ligation buffer, 5 ng/μl of pT-Adv vector and 4 U/μl of T4 DNA ligase for ligation. The mixture was then incubated at 14°C overnight. For TOPO TA cloning kits, 1 μl of reamplified PCR product was mixed up with 0.2 M NaCl, 0.01 M $MgCl_2$ and 0.17 ng/μl pCR®II-TOPO® vector. The mixture was then incubated at room temperature for 5 min.

2.12.2.2 Transformation

Two microliters of ligation products were then mixed with TOP 10F' *E. coli* competent cells and chilled on ice for 30 min. The mixture was then incubated in 42°C water bath for 90 sec, chilled on ice immediately for 2 min. and then diluted to 1

ml by LB medium. Then the mixture was incubated at 37°C with shaking for 1 hr and centrifuged at 2,500 rpm at 4°C for 8 min. The supernatant was discarded and the cell pellet was resuspended with 300 μl LB medium. The transformed cells were spreaded on LB agar plates containing 100 μg/ml ampicillin, 40 μl of 100 mM IPTG and 40 μl of 40 mg/ml X-Gal. The plates were incubated at 37°C incubator for 16 hr.

2.12.2.3 Phenol-chloroform extraction

Single white colony was incubated in 3 ml LB medium containing 100 μg/ml of ampicillin at 37°C for 16 hours with shaking. Thirty microliters of bacterial culture were mixed with 30 μl of phenol: chloroform: isoamyl alcohol (25:24:1) for screening recombinant clones. The mixture was vortexed vigorously and then centrifuged at 14,000 rpm at room temperature for 2 min. Ten microliters of the supernatant were resolved on 0.6% agarose, 0.5X TBE gels with ethidium bromide staining.

2.12.2.4 Confirmation of insert size by *Eco*RI digestion

The recombinant clones which showed positive result in the phenol-cholorform extraction were selected for *Eco*RI digestion for further confirmation of the insert sizes. One milliliter of bacterial culture was centrifuged at 14,000 rpm at 4°C for 1 min. The supernatant was discarded and the cell pellet was resuspended with 17 μl autoclaved distilled water containing 250 μg/ml RNase A. The resuspended cells were boiled for 1 min and then centrifuged at 14,000 rpm at room temperature for 1 min. Seventeen microliters of supernatant were digested with 10 units of *Eco*RI in 1X *Eco*RI buffer at 37°C for 2 hr. The mixture was then resolved on 1% or 2% agarose, 0.5X TBE gels with ethidium bromide staining.

2.12.2.5 Mini-preparation of plasmid DNA from recombinant clones

Plasmid DNA of cDNA fragments was prepared for by QIAGEN® spin miniprep kit. Ten milliliters of bacterial culture were centrifuged at 2,500 rpm at 4°C for 10 min. The supernatant was discarded and cell pellet was resuspended in 250 μl of buffer P1 containing 100 μg/ml RNase A. The resuspended cells were then mixed with 250 μl of buffer P2 by inverting several times. The mixture was incubated at room temperature for about 5 min. Three hundred and fifty microliters of buffer P3 were added to the mixture and mixed by inverting several times. The mixture was centrifuged at 14,000 rpm at room temperature for 15 min. The supernatant was transferred to QIAprep spin columns in 2 ml collection tubes and centrifuged at 14,000 rpm for 1 min. The flow-through was discarded and the columns were washed with 750 μl buffer PE. The columns were transferred to new 1.5 ml eppendrof tube. The plasmid DNA was eluted by adding 50 μl of nuclease free water and centrifuged at 14,000 rpm at room temperature for 1 min twice. The samples were diluted to 6 μl with sterile water and stored at 4°C.

2.13 Sequencing of subcloned cDNA fragments

After confirming the sizes of the cDNA fragments, sequencing was performed by using CEQ dye terminator cycle sequencing kit, M13 forward (-20) (5'-GTAAAACGACGGCCAG-3') and M13 reverse sequencing primers (5'-CAGGAAACAGCTATGAC-3').

2.13.1 Materials

CEQ dye terminator cycle sequencing kit was purchased from Beckman (USA). EDTA, sterile water and sodium acetate were purchased from Sigma (USA). M13 forward (-20) and M13 reverse primers were obtained from Invitrogen (USA).

2.13.2 Methods

2.13.2.1 Sequencing of fluoroDD cDNA fragments

DNA sequencing reaction was prepared by mixing 100 fmol dsDNA in 6 µl sterile water with 2 µl of 10X sequencing reaction buffer, 1 µl of dNTP mix, 2 µl of ddUTP dye terminator, 2 µl of ddGTP dye terminator, 2 µl of ddCTP dye terminator, 2 µl of ddATP dye terminator, 2 µl of 1.6 µM M13 forward (-20) or M13 reverse primer and 1 µl of Taq polymerase in a 0.2 ml thin-wall PCR tube (Appendix A, Table A6). The mixture was mixed by finger-tapping and spinned down. PCR was performed in a thermal cycler (GeneAmp® PCR system 9700) and the PCR program was as follows: 30 cycles of 96°C for 20 sec, 50°C for 20 sec, 60°C for 4 min and hold at 4°C. Stop solution (final concentration of 1.5 M sodium acetate and 50 mM EDTA) was freshly prepared and 4 µl stop solution was added to new 1.5 ml eppendrof tube. The mixture was transferred to a new 1.5 ml eppendrof tube and 1 µl of 20 mg/ml of glycogen was added to each eppendrof tube. The mixture was vortexed vigorously and 60 µl of cold 95% ethanol were added to the mixture and vortexed. The samples were then centrifuged at 14,000 rpm at 4°C for 20 min and the supernatant was discarded carefully to avoid disturbing the pellet. The pellet was washed with 200 µl cold 70% ethanol and then centrifuged immediately at 14,000 rpm at 4°C for 25 min.

The supernatant was discarded carefully and the washing steps were repeated. The DNA pellet was vacuum dried for at least 45 min until all solvent was evaporated. Forty microliters of sample loading buffer (from the Beckman sequencing kit) were added to the DNA pellet. The DNA pellet was rehydrated for 30 min and then resuspended by pipetting up and down. The resuspended DNA samples were transferred to a 96-well polypropylene sample plate recommended for the CEQ automated DNA sequencer (Beckman). One drop of light mineral oil (provided by the sequencing kit) was added to each sample. The sample plate was loaded into the CEQ DNA sequencer and DTCS-2 method was used for dsDNA plasmid sequencing using long capillary column.

2.13.2.2 BLAST search against computer database

After DNA sequencing, sequence homology was performed by searching the computer database of Basic Local Alignment Search Tool of non-redundant, mouse and human ESTs (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

2.14 Northern blot analysis of sequenced cDNA fragments

After sequencing and sequence homology searching, the differential display patterns of fluoroDD were further confirmed by Northern blot analysis. The plasmid DNA used for sequencing was used for the preparation of the probe for Northern blot anaylsis. DIG labeled RNA probe and PCR-DIG labeled cDNA probe were prepared.

2.14.1 Materials

DIG Easy Hyb, Tween 20, blocking reagent, anti-DIG-alkaline phosphatase antibody, DIG-labeled RNA molecular weight marker I (0.39-6.9 kb), DIG RNA labeling mix, T7 polymerase, PCR-DIG labeling mix, nitro blue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) were obtained from Roche (Germany). Agarose, MOPS and sodium chloride were purchased from USB (USA). Magnesium chloride, PCR buffer and Taq polymerase were from GenSys (UK). Formaldehyde (37%) was obtained from Riedel-de Haën (Germany). Biodyne® membrane was purchased from Pall (USA). Gel blotting paper was obtained from Schleicher & Schuell (Germany). Three MM filter paper was purchased from Whatman® (UK). Kapak SealPAK pouches were from KAPAK® (USA). Ethidium bromide, maleic acid and sodium citrate were purchased from Sigma (USA).

2.14.2 Methods

2.14.2.1 Formaldehyde agarose gel electrophoresis of total RNA

Three mice from each treatment groups including PPARα (+/+) control, PPARα (+/+) 0.1% Wy-14,643, PPARα (-/-) control and PPARα (-/-) 0.1% Wy-14,643 were used for Northern blot analysis. For temporal study of gene expression, two mice from each treatment groups at 24 hr, 1 wk, 2 wk, 6 mo and 11 mo were used for Northern blot analysis. Total RNA (20 or 30 μg in 10 μl of formamide) samples and 10 μl RNA molecular marker were mixed with 2 μl of 10X MOPS, 3 μl of 37% formaldehyde, 2 μl of loading dye and 5 μl of nuclease-free water. RNA samples were then denatured by heating at 68°C for 15 min and then chilled on ice immediately. RNA samples were then separated on a 1% formaldehyde-agarose gel at

30 V for about 15 hr. For temporal study of gene expression, the RNA samples of the 5 different treatment time points were resolved on two gels: PPARα (+/+) control and PPARα (+/+) 0.1% Wy-14,643 on one gel, while PPARα (-/-) control and PPARα (-/-) 0.1% Wy-14,643 on another gel. The RNA gel was post-stained with 0.5 μg/μl ethidium bromide for 45 min and wash with distilled water for 20 min 3 times. After taking photos, RNA gel was washed in 10X SSC for 30 min and then transferred to a positively charged nylon membrane in 10X SSC buffer by capillary action for 24 hr. After washing with 5X SSC for 5 min, the membrane was baked for 2 hrs at 80°C for fixing the RNA onto the membrane.

2.14.2.2 Preparation of DIG-labeled RNA probes for hybridization

Two nanograms of plasmid DNA were mixed with 80 μM dNTP mix (1:1:1:1), 0.2 μM AP, 0.2 μM T7 ARP (same the primer pairs used for running the fluoroDD RT-PCR), 1X PCR buffer and 0.05 U/μl of *Taq* polymerase (Appendix A, Table A7). PCR reaction was performed in a thermal cycler (GeneAmp®PCR system 9700) and the program was as follows: 95°C for 2 min, followed by 29 cycles of 92°C for 30 sec, 60°C for 30 sec, 72°C for 2 min and 72°C for 7 min and hold at 4°C. Two microliters of reamplified PCR product were resolved on 1% or 2 % agarose, 0.5X TBE with ethidium bromide staining. After confirming the size and specificity of the PCR product, 1 μg of PCR product was used for DIG RNA labeling. For each reaction, PCR product was mixed with 1 mM of DIG RNA labeling mix, 1X transcriptional buffer and 2 U/μl of T7 polymerase. The mixture was mixed by finger-tapping and incubated at 37°C for 2 hr. The labeling was stopped by adding 2 μl of 0.2 M EDTA at pH 8.0. One microliter labeled probe was resolved on 1% or 2% agarose, 0.5X

TBE gel with ethidium bromide. The labeled probe was stored at -80°C or used immediately.

2.14.2.3 Preparation of PCR-DIG labeled cDNA probe for hybridization

Twenty nanograms of plasmid DNA were mixed with 200 μM PCR DIG labeling mix (1:1:1:1), 1 μM AP, 1 μM T7 ARP (same the primer pairs used for running the fluoroDD RT-PCR), 1X PCR buffer and 0.04 U/μl of *Taq* polymerase (Appendix A, Table A8). PCR reaction was performed in a thermal cycler (GeneAmp®PCR system 9700) and the program was as follows: 95°C for 7 min, followed by 30 cycles of 95°C for 45 sec, 60°C for 1 min, 72°C for 2 min and 72°C for 7 min and hold at 4°C. One microliter of PCR-DIG labeled cDNA probe was resolved on 1% or 2 % agarose, 0.5X TBE with ethidium bromide staining. After confirming the size and specificity of the PCR DIG-labeled cDNA probe, the labeled probe was stored at -80°C or used immediately.

2.14.2.4 Hybridization and colour development

The membrane was pre-hybridized with 20 ml DIG Easy Hyb buffer in a sealable bag at 42 °C for PCR DIG-labeled cDNA probe or 60°C for DIG-labeled RNA probe for 2 hr. For RNA probe, the labeled probe was added to the pre-hybridized buffer and mixed well in a 50 ml Falcon tube. For PCR DIG-labeled probe, the probe was first denatured by boiling for 10 min and then chilled on ice immediately. The denatured probe was added to the pre-hybridized buffer and mixed well in a 50 ml Falcon tube. The pre-hybridized buffer containing the labeled probe was added to the seablable bags and hybridized for overnight at 42 °C for PCR DIG-

labeled cDNA probe or 60°C for DIG-labeled RNA probe. For temporal study of gene expression, two membranes of PPARα (+/+) and PPARα (-/-) control and 0.1% Wy-14,643 groups were pre-hybridized and hybridized in 40 ml DIG-Easy Hyb buffer back to back in a sealable bag. The hybridized membrane was washed in 2X SSC (3 M sodium chloride and 0.3 M sodium citrate, pH 7.0) / 0.1% SDS at room temperature for 5 min twice and in either 0.1X SSC / 0.1% SDS at 68°C or 0.5X SSC / 0.1% SDS at 60°C for 5 min twice. The membrane was washed in washing buffer [0.3% Tween 20 in maleic acid buffer (pH 7.5)] for 5 min and then incubated with 1X blocking solution [1X blocking solution in maleic acid buffer (pH 7.5)] for 1 hr. After washing, the membrane was incubated with anti-DIG-alkaline phosphatase antibody in a final concentration of 0.075 U/ml of 1X blocking solution for 1 hr. The membrane was washed in washing buffer twice and then equilibrated in detection buffer [0.1 M Tris-HCl (pH 9.5), 0.1 M $MgCl_2$ 0.1 M NaCl] for 5 min. The membrane was allowed to develop colour in 25 ml of detection buffer containing 125 µl and 93.8 µl of NBT and BCIP, respectively.

Chapter 3 Results

3.1 Confirmation of genotypes by PCR

As there are no observable phenotypic differences between the PPARα (+/+) and PPARα (-/-) mice, the genotypes of these mice were confirmed before animal feeding. The sizes of the PCR products of PPARα (+/+) and PPARα (-/-) mice were 191 and 1248 bp, respectively (Figures 3.1.1-3.1.2), which was matched with the results from the previous study (Lee *et al.*, 2002).

3.2 Body weight changes

Body weight was significantly lower in the PPARα (+/+) fed with 0.1% Wy-14,643 compared to the controls (6 and 11 months feeding experiments). In Figure 3.2.1, lower body weight was shown in 0.1% Wy-14,643 treated PPARα (+/+) mice when compared to the control fed PPARα (+/+) mice since 1 week feeding. After 6 months of feeding, there was a 9% decrease in body weight when compared with the original body weight. However, increase in body weight in the PPARα (+/+) mice fed with control diet, PPARα (-/-) mice fed with control and 0.1% Wy-14,643 were observed (21%, 17% and 21% of the original body weights, respectively). A significant decrease in body weight of 0.1% Wy-14,643 fed PPARα (+/+) mice was also observed in the 11 months feeding on 0.1% Wy-14,643 diet (Figure 3.2.2). After 11 months feeding, there was a 14% decrease in body weight in the 0.1% Wy-14,643 fed PPARα (+/+) mice. When compared with the body weight before starting the experiment, there were 23%, 22 % and 17% increase in body weight for the PPARα

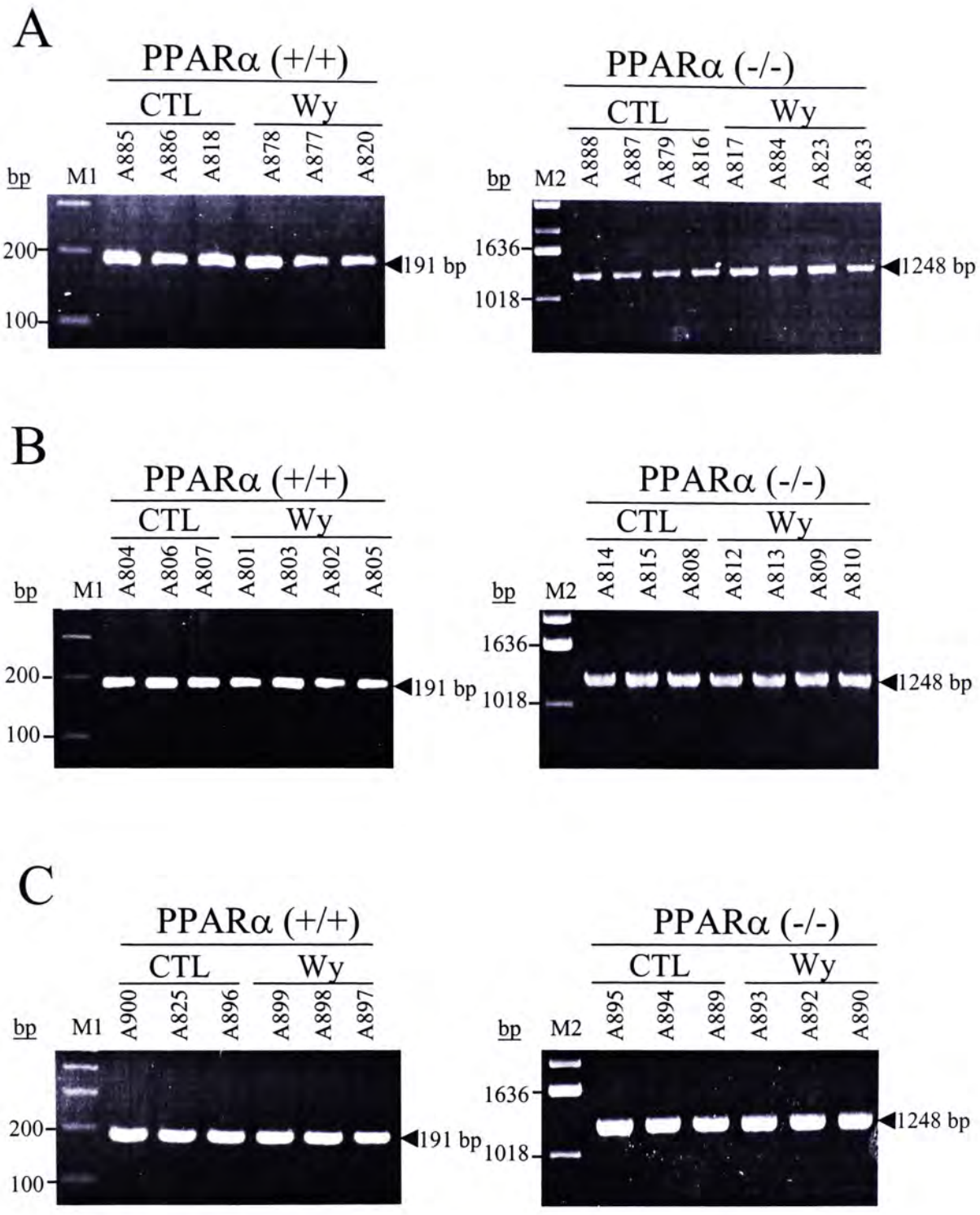

Figure 3.1.1. Confirmation of genotypes of PPARα (+/+) and PPARα (-/-) mice used for (A) 24 hours, (B) 1 week and (C) 2 weeks treatment. Tail genotypings of PPARα (+/+) and PPARα (-/-) mice fed with a control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week and 2 weeks were performed. The PCR products of PPARα (+/+) and PPARα (-/-) mice were resolved on 2% and 1% agarose, 0.5X TBE gels with ethidium bromide staining, respectively. The expected sizes of PCR products of PPARα (+/+) and PPARα (-/-) mice were 191 and 1248 bp, respectively. M1, 100 bp DNA marker; M2, 1 kb DNA marker.

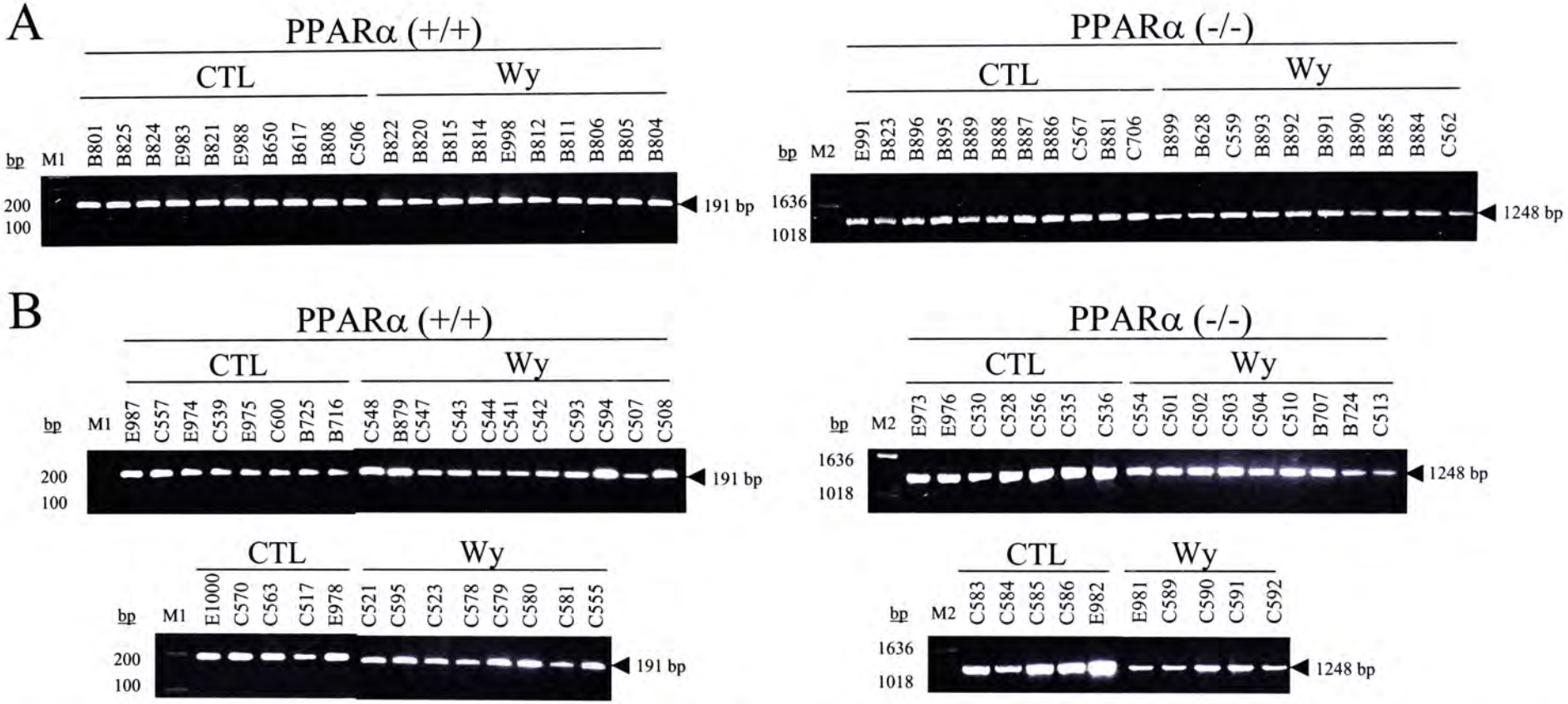

Figure 3.1.2. Confirmation of genotypes of PPARα (+/+) and PPARα (-/-) mice used for (A) 6 months and (B) 11 months treatment. Tail genotypings of PPARα (+/+) and PPARα (-/-) mice fed with a control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 6 months and 11 months were performed. The PCR products of PPARα (+/+) and PPARα (-/-) mice were resolved on 2% and 1% agarose, 0.5X TBE gels with ethidium bromide staining, respectively. The expected sizes of PCR products of PPARα (+/+) and PPARα (-/-) mice were 191 and 1248 bp, respectively. M1, 100 bp DNA marker; M2, 1 kb DNA marker.

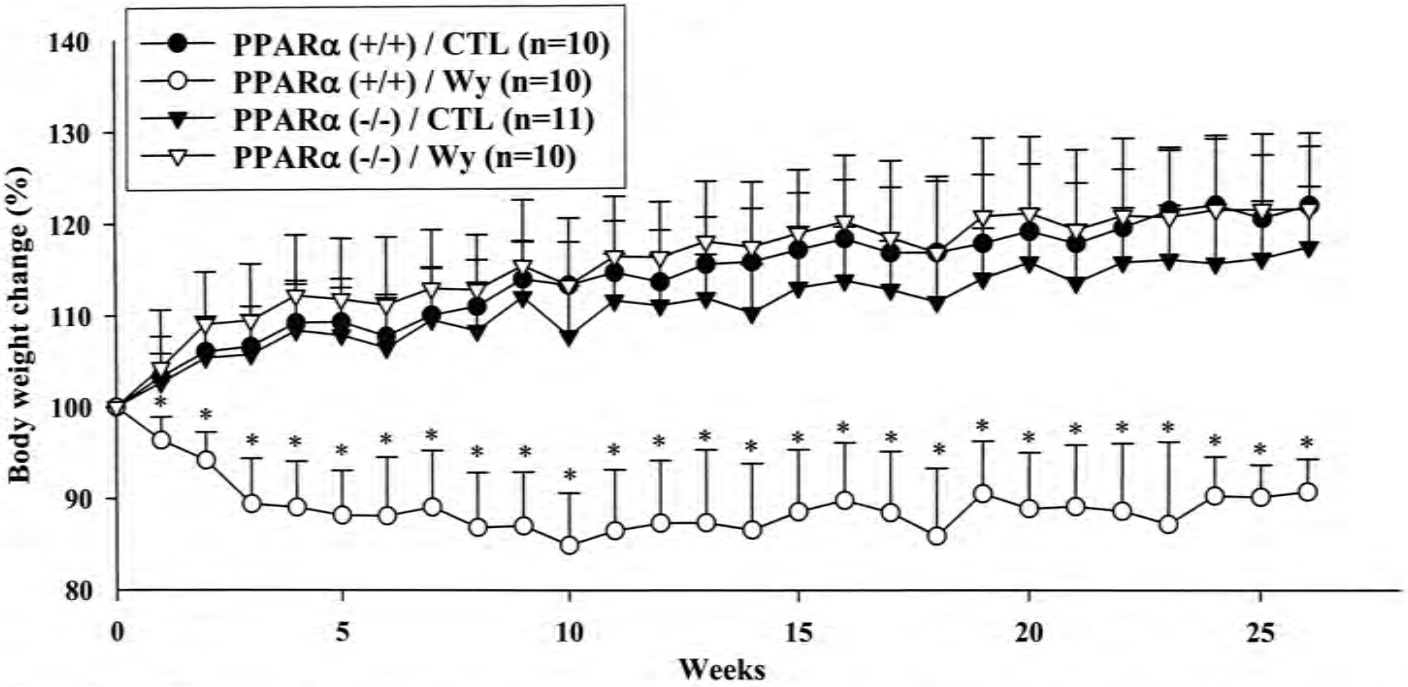

Figure 3.2.1. Effect 6 months of 0.1% Wy-14643 treatment on body weight change (%) in PPARα (+/+) and PPARα (-/-) mice. Data are expressed as mean ± SD (n ≥10). Two-way ANOVA was used to compare the differences in mean values within each time-point using SigmaStat Program. *Significant differences ($p \leq 0.001$) from control diet (CTL) group in PPARα (+/+), control diet (CTL) and 0.1% Wy-14,643 treated PPARα (-/-) mice were started from week 1 and continued till the end of 6 months experiment.

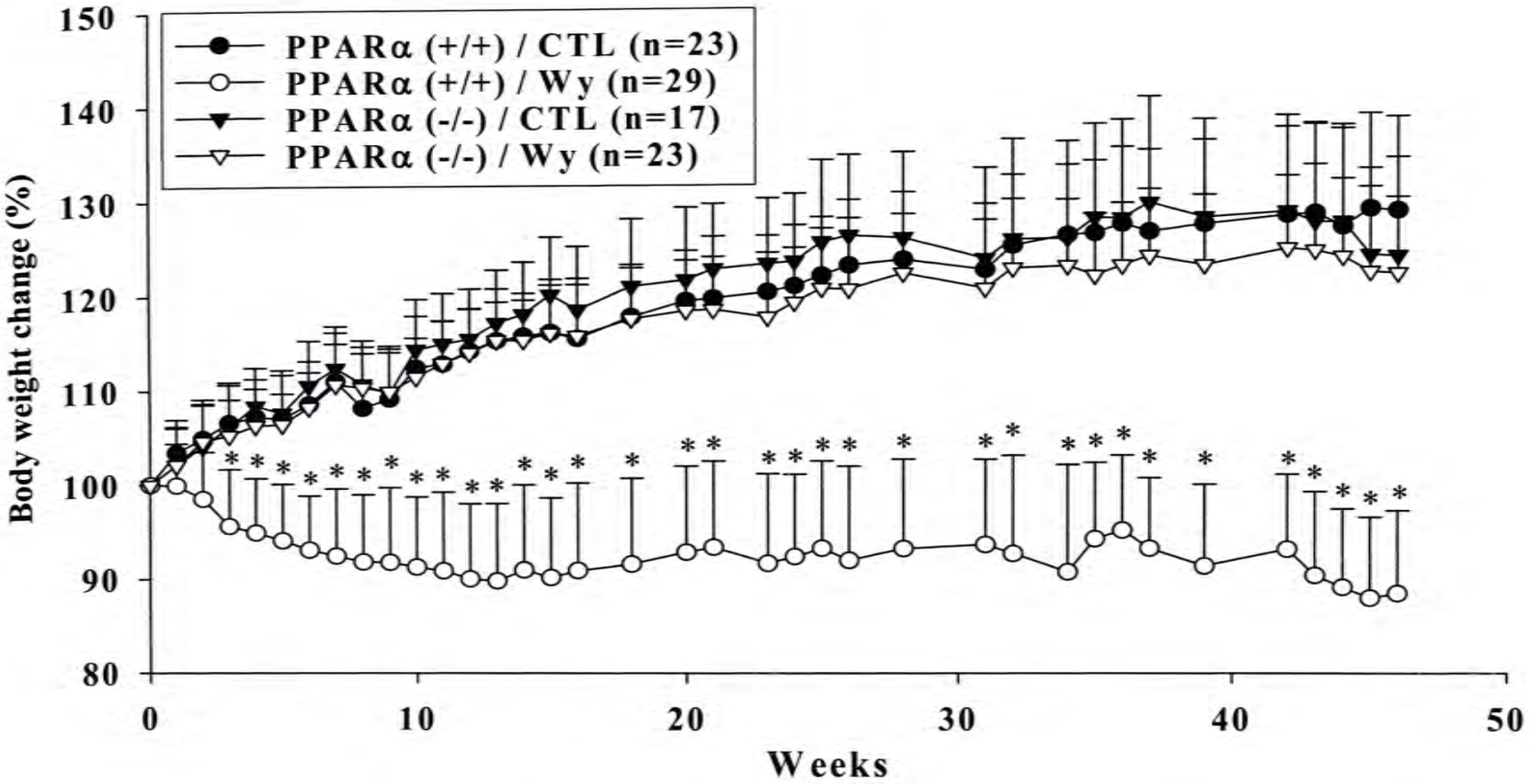

Figure 3.2.2. Effect of 11 months 0.1% Wy-14643 treatment on body weight change (%) in PPARα (+/+) and PPARα (-/-) mice. Data are expressed as mean ± SD (n ≥17). Two-way ANOVA was used to compare the differences in mean values within each time-point using SigmaStat Program. *Significant differences ($p \leq 0.001$) from control diet (CTL) group in PPARα (+/+), control diet (CTL) and 0.1% Wy-14,643 treated PPARα (-/-) mice were started from week 1 and till the end of 11 months treatment.

(+/+) mice fed with control diet, PPARα (-/-) mice fed with control and 0.1% Wy-14,643, respectively. The lower body weight of 0.1% Wy-14,643 treatment of PPARα (+/+) mice when compared with the other three treatment groups was matched with the previous study (Peters *et al.*, 1997).

Some of the PPARα (+/+) mice treated with the 0.1% Wy-14,643 diet died before the end of the 6 and 11 months feeding experiment. The high mortality rate observed in PPARα (+/+) mice fed with 0.1% Wy-14,643 treatment was also reported in the previous study (Peters *et al.*, 1997). Two out of ten mice died (after weeks 13 and 24) in the 6 months feeding experiment. The mortality rate was increased with prolonged treatment. Fifty-two percent of the PPARα (+/+) mice fed with 0.1% Wy-14,643 started to in the 11 months feeding experiment. The high mortality in PPARα (+/+) mice fed with 0.1% Wy-14,643 might be due to the enlargement of liver and liver tumor formation.

3.3 Organ weight changes

A time-dependent increase in liver weight was observed in PPARα (+/+) mice fed with the 0.1% Wy-14,643 for 24 hours, 1 week, 2 weeks, 6 months and 11 months (Figure 3.3.1). The liver weights (% of body weight) of the PPARα (+/+) mice fed with the 0.1% Wy-14,643 diet for 24 hours (1.14-fold), 1 week (1.56-fold), 2 weeks (2.23-fold), 6 months (3-fold) and 11 months (3.88-fold) higher than the PPARα (+/+) mice fed with control diet. However, liver enlargement was not observed in the 0.1% Wy-14,643-treated PPARα (-/-) mice at all time points investigated. This observation was agreed to the well-characterized phenomenon of peroxisome proliferators-induced hepatomegaly.

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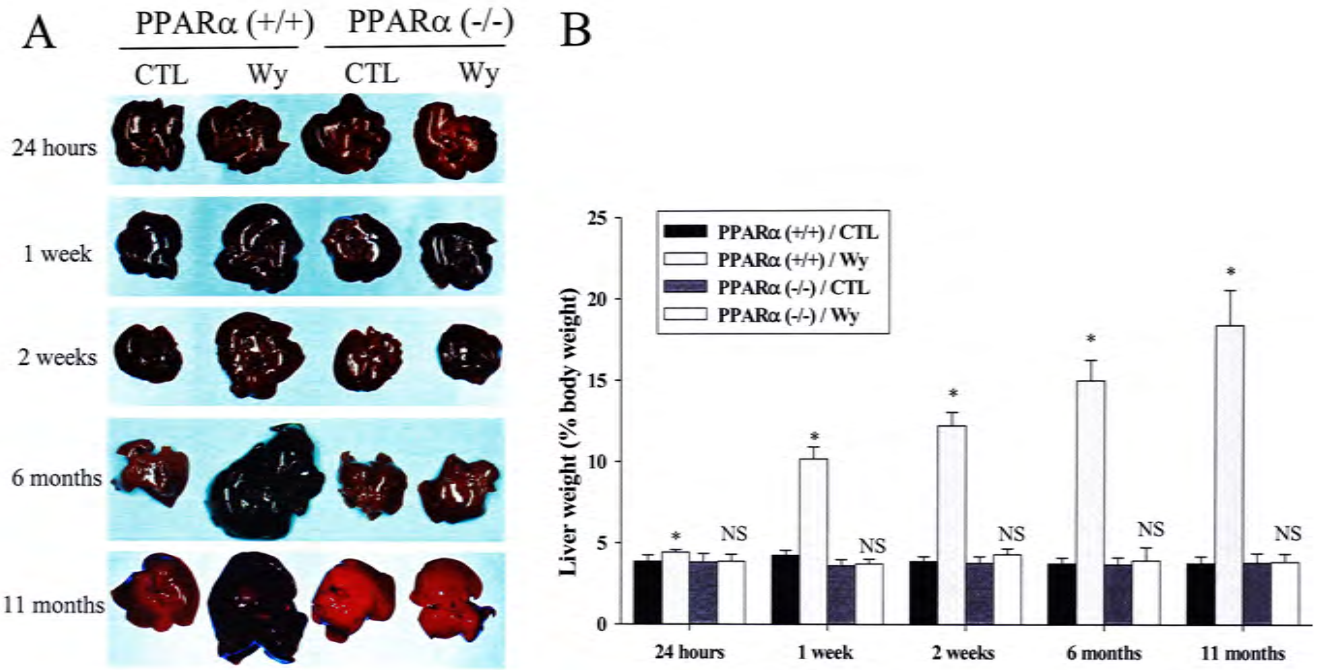
Figure 3.3.1. Effect of 24 hours, 1 week, 2 weeks, 6 months and 11 months 0.1% Wy-14643 treatment on (A) liver morphology and (B) liver weights of PPARα (+/+) and PPARα (-/-) mice. Time-dependent increase in liver weights was observed in PPARα (+/+) mice fed with a 0.1% Wy-14,643 diet for 24 hr, 1 wk, 2 wk, 6 mo and 11 mo. To account for change in body weights after fed with 0.1% Wy-14,643, liver weights were normalized as percentage of body weight. Data are expressed as mean ± SD (n ≥8). ANOVA on ranks was used to compare the differences in mean values within each time-point using SigmaStat Program. *Significant difference ($p \leq 0.05$) from control diet (CTL) group in PPARα (+/+) mice. NS, no significant difference.


A
PPARα (+/+)
PPARα (-/-)
CTL
Wy
CTL
Wy
24 hours
1 week
2 weeks
6 months
11 months
B
PPARα (+/+) / CTL
PPARα (+/+) / Wy
PPARα (-/-) / CTL
PPARα (-/-) / Wy
Liver weight (% body weight)
25
20
15
10
5
0
*
NS
24 hours
1 week
2 weeks
6 months
11 months

In contrast to the liver weight, there was a time-dependent decrease in white fat weight PPARα (+/+) mice fed with the 0.1% Wy-14,643 diet for 2 weeks (47%), 6 months (75%) and 11 months (86%) (Figure 3.3.2) compared with their corresponding controls. However, this time-dependent decrease in white fat weight was not observed in the 0.1% Wy-14,643-treated PPARα (-/-) mice when compared to the controls. The decrease in white fat weight was due to the well-documented hypolipidemic effect of the Wy-14,643 compound.

Similar to the white fat weight, a significant time-dependent decrease in the relative brown fat weight was also found in the PPARα (+/+) mice fed with the 0.1% Wy-14,643 diet for 2 weeks, 6 months and 11 months (Figure 3.3.3). The decrease in brown fat weight for 2 weeks, 6 months and 11 months was 29%, 53% and 62%, respectively and was due to the hypolipidemic action of Wy-14,643 compound.

There was a significant decrease in spleen weight in PPARα (+/+) mice following 11 months Wy-14,643 treatment (48%) (Figure 3.3.4). The decrease in size of spleen was reported in a previous study (Yang *et al.*, 2000).

No significant change in the brain, heart, lung and testis weights observed in both PPARα (+/+) and (-/-) mice fed the Wy-14,643 diet at all time points studied compared with their controls (Table 3.3.1).

3.4 Serum cholesterol and triglyceride levels

Previous studies have shown that Wy-14,643 treatment decreased the serum cholesterol and triglyceride levels in PPARα (+/+) mice (Issemann and Green, 1990). To determine the hypolipidemic effect of Wy-14,643, the serum cholesterol and triglyceride levels of all the treatment groups at different feeding treatments were

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Figure 3.3.2. Effect of 24 hours, 1 week, 2 weeks, 6 months and 11 months 0.1% Wy-14643 treatment on (A) white fat morphology and (B) white fat weights of PPARα (+/+) and PPARα (-/-) mice. Time-dependent decrease in white fat weights was observed in PPARα (+/+) mice fed with 0.1% Wy-14,643 diet for 2 wk, 6 mo and 11 mo. To account for change in body weights after fed with a 0.1% Wy-14,643, white fat weights were normalized as percentage of body weight. Data are expressed as mean ± SD (n ≥8). ANOVA on ranks was used to compare the differences in mean values within each time-point using SigmaStat Program. *Significant difference ($p \leq 0.05$) from control diet (CTL) group in PPARα (+/+) mice. NS, no significant difference.

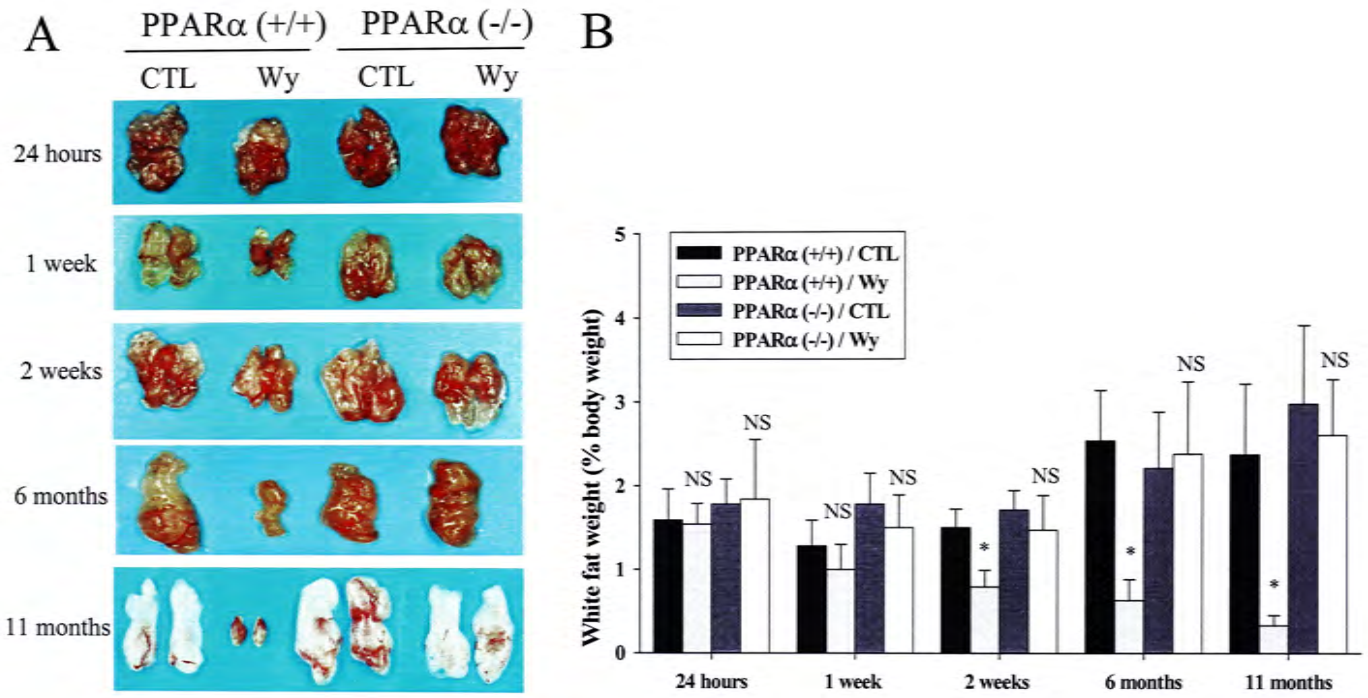
A
PPARα (+/+)
PPARα (-/-)
CTL
Wy
CTL
Wy
24 hours
1 week
2 weeks
6 months
11 months
B
PPARα (+/+) / CTL
PPARα (+/+) / Wy
PPARα (-/-) / CTL
PPARα (-/-) / Wy
White fat weight (% body weight)
0
1
2
3
4
5
NS
*
24 hours
1 week
2 weeks
6 months
11 months

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Figure 3.3.3. Effect of 24 hours, 1 week, 2 weeks, 6 months and 11 months 0.1% Wy-14643 treatment on (A) brown fat morphology and (B) brown fat weights of PPARα (+/+) and PPARα (-/-) mice. Time-dependent decrease in brown fat weights was observed in PPARα (+/+) mice fed with 0.1% Wy-14,643 diet for 2 wk, 6 mo and 11 mo. To account for change in body weights after fed with a 0.1% Wy-14,643, brown fat weights were normalized as percentage of body weight. Data are expressed as mean ± SD (n ≥8). ANOVA on ranks was used to compare the differences in mean values within each time-point using SigmaStat Program. *Significant difference ($p \leq 0.05$) from control diet (CTL) group in PPARα (+/+) mice. NS, no significant difference.

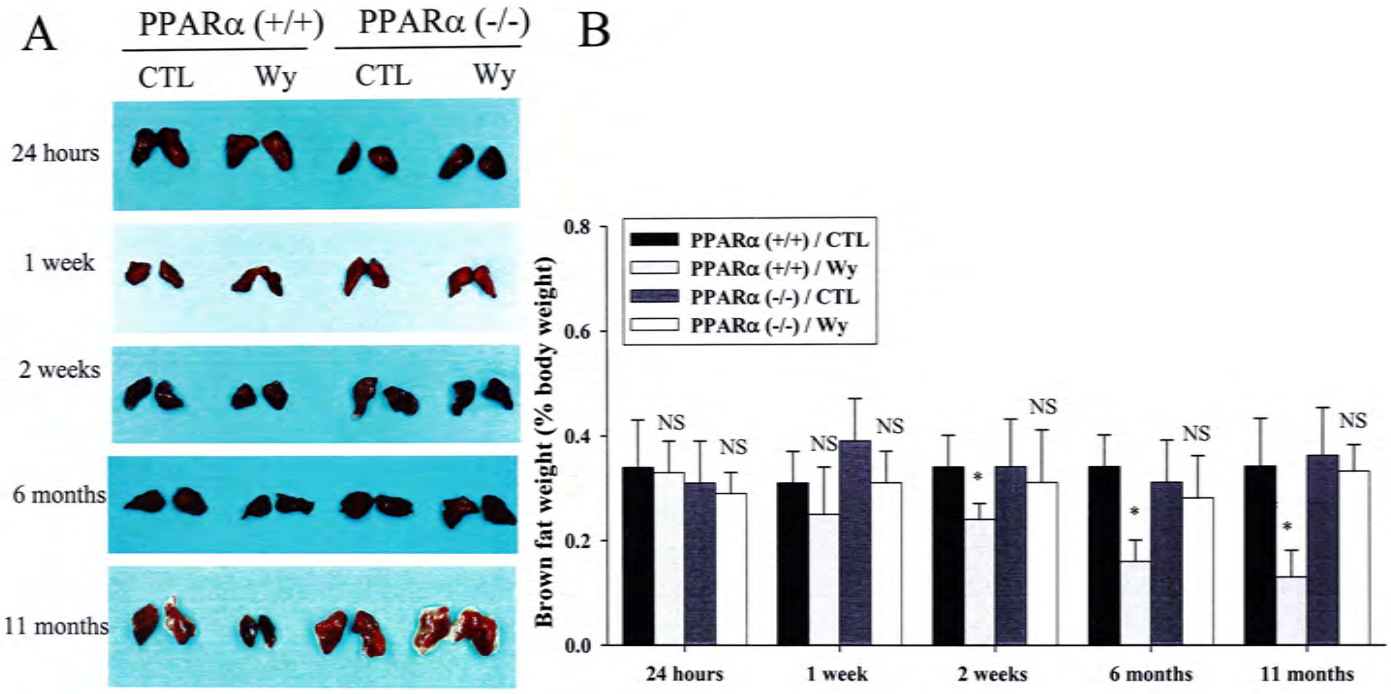
A
PPARα (+/+)
PPARα (-/-)
CTL
Wy
CTL
Wy
24 hours
1 week
2 weeks
6 months
11 months
B
PPARα (+/+) / CTL
PPARα (+/+) / Wy
PPARα (-/-) / CTL
PPARα (-/-) / Wy
Brown fat weight (% body weight)
0.0
0.2
0.4
0.6
0.8
NS
*
24 hours
1 week
2 weeks
6 months
11 months

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Figure 3.3.4. Effect of 24 hours, 1 week, 2 weeks, 6 months and 11 months 0.1% Wy-14643 treatment on (A) spleen morphology and (B) spleen weights of PPARα (+/+) and PPARα (-/-) mice. Decrease in spleen weights was observed in PPARα (+/+) mice fed with 0.1% Wy-14,643 diet for 11 mo. To account for change in body weights after fed with a 0.1% Wy-14,643, spleen weights were normalized as percentage of body weight. Data are expressed as mean ± SD (n ≥8). ANOVA on ranks was used to compare the differences in mean values within each time-point using SigmaStat Program. *Significant difference ($p \leq 0.05$) from control diet (CTL) group in PPARα (+/+) mice. NS, no significant difference.

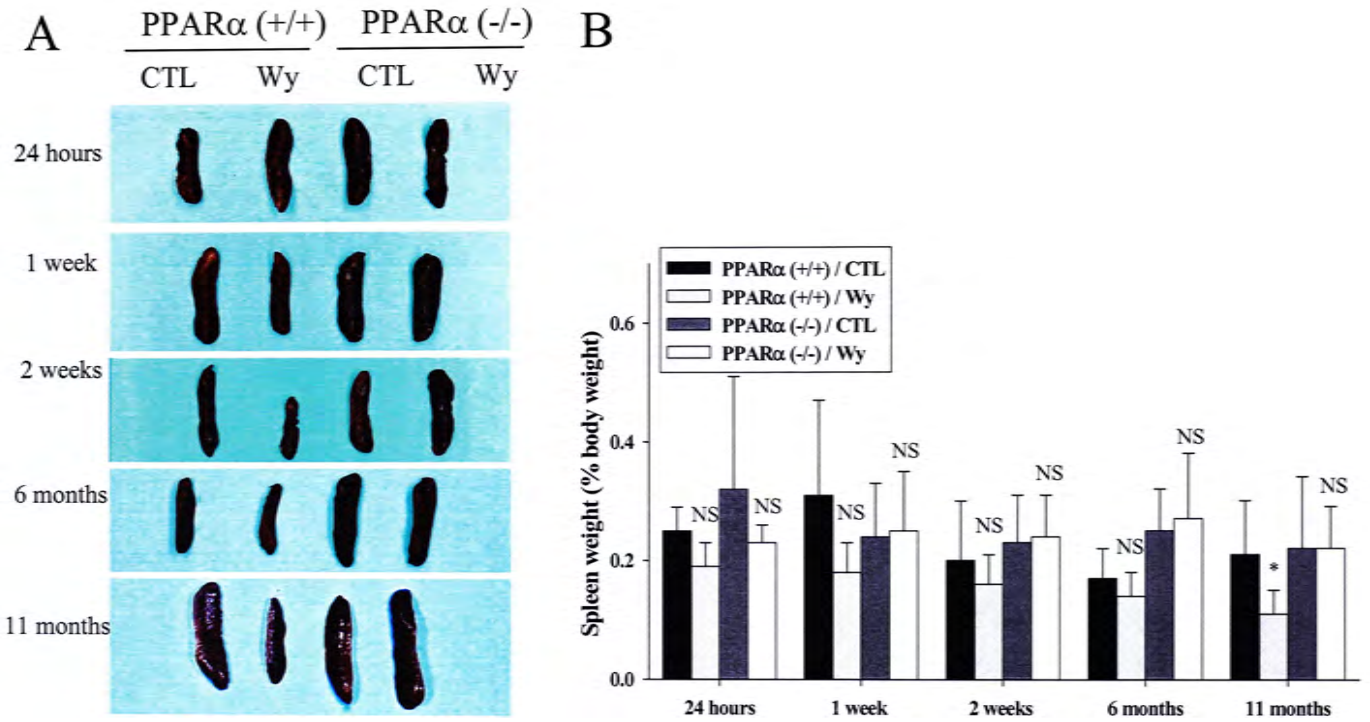
A
PPARα (+/+)
PPARα (-/-)
CTL
Wy
CTL
Wy
24 hours
1 week
2 weeks
6 months
11 months
B
PPARα (+/+) / CTL
PPARα (+/+) / Wy
PPARα (-/-) / CTL
PPARα (-/-) / Wy
Spleen weight (% body weight)
0.0
0.2
0.4
0.6
NS
NS
NS
NS
NS
NS
NS
NS
NS
NS
*
24 hours
1 week
2 weeks
6 months
11 months

Table 3.3.1. Effect of Wy-14,643 feeding on kidney, heart, brain, lung and testis weight change of 24 hours, 1 week, 2 weeks, 6 months and 11 months feeding experiment.

	Group	Kidney (%)	Heart (%)	Brain (%)	Lung (%)	Testis (%)
24 hr	PPARα (+/+)/CTL	1.48 ± 0.13	0.54 ± 0.04	1.63 ± 0.19	0.65 ± 0.11	0.94 ± 0.14
	PPARα (+/+)/Wy	1.49 ± 0.15	0.54 ± 0.07	1.73 ± 0.19	0.71 ± 0.11	0.74 ± 0.09
	PPARα (−/−)/CTL	1.45 ± 0.16	0.58 ± 0.07	1.66 ± 0.15	0.62 ± 0.09	0.79 ± 0.04
	PPARα (−/−)/Wy	1.55 ± 0.38	0.59 ± 0.07	1.51 ± 0.15	0.67 ± 0.23	0.79 ± 0.06
1 wk	PPARα (+/+)/CTL	1.55 ± 0.13	0.56 ± 0.08	1.76 ± 0.31	0.72 ± 0.23	0.86 ± 0.04
	PPARα (+/+)/Wy	1.60 ± 0.16	0.59 ± 0.12	2.04 ± 0.13	0.78 ± 0.24	0.94 ± 0.06
	PPARα (−/−)/CTL	1.51 ± 0.21	0.55 ± 0.11	1.53 ± 0.07	0.74 ± 0.23	1.08 ± 0.36
	PPARα (−/−)/Wy	1.54 ± 0.23	0.56 ± 0.10	1.64 ± 0.14	0.91 ± 0.25	0.74 ± 0.06
2 wk	PPARα (+/+)/CTL	1.51 ± 0.09	0.53 ± 0.03	1.74 ± 0.06	0.81 ± 0.16	0.87 ± 0.23
	PPARα (+/+)/Wy	1.60 ± 0.09	0.55 ± 0.05	1.92 ± 0.12	0.71 ± 0.15	0.79 ± 0.12
	PPARα (−/−)/CTL	1.43 ± 0.12	0.51 ± 0.07	1.65 ± 0.17	0.69 ± 0.10	0.90 ± 0.38
	PPARα (−/−)/Wy	1.51 ± 0.18	0.53 ± 0.04	1.48 ± 0.14	0.65 ± 0.16	0.73 ± 0.05
6 mo	PPARα (+/+)/CTL	1.51 ± 0.10	0.50 ± 0.06	1.44 ± 0.14	0.67 ± 0.13	0.66 ± 0.10
	PPARα (+/+)/Wy	1.47 ± 0.11	0.71 ± 0.06	1.90 ± 0.16	0.95 ± 0.21	0.73 ± 0.05
	PPARα (−/−)/CTL	1.58 ± 0.19	0.56 ± 0.06	1.47 ± 0.18	0.77 ± 0.17	0.68 ± 0.13
	PPARα (−/−)/Wy	1.57 ± 0.21	0.52 ± 0.06	1.43 ± 0.16	0.71 ± 0.14	0.67 ± 0.16
11 mo	PPARα (+/+)/CTL	1.62 ± 0.13	0.54 ± 0.07	1.43 ± 0.15	0.63 ± 0.11	0.68 ± 0.07
	PPARα (+/+)/Wy	1.50 ± 0.17	0.75 ± 0.10	1.90 ± 0.22	0.93 ± 0.16	0.64 ± 0.08
	PPARα (−/−)/CTL	1.57 ± 0.18	0.54 ± 0.05	1.35 ± 0.18	0.61 ± 0.10	0.60 ± 0.14
	PPARα (−/−)/Wy	1.54 ± 0.11	0.53 ± 0.05	1.42 ± 0.12	0.61 ± 0.09	0.64 ± 0.14

Relative body weight was expressed as organ weight (g)/ body weight (g) X 100%.

measured in this study. There were no significant differences in serum cholesterol level among the four treatment groups at 24 hours gavage, 1 week, 2 weeks and 6 months feeding (Figure 3.4.1). There was a 29% increase in serum cholesterol level in PPARα (+/+) mice fed the Wy-14,643 diet for 11 months when compared with the PPARα (+/+) mice fed the control diet. This increase was not observed in the PPARα (-/-) mice fed with 0.1% Wy-14,643. In contrast, a time-dependent decrease in serum triglyceride level was observed in the PPARα (+/+) mice fed with the 0.1% Wy-14,643 diet for 24 hours (44%), 1 week (38%), 2 weeks (35%), 6 months (66%) and 11 months (77%) (Figure 3.4.2) feeding studies were observed. However, no significant changes were found in the PPARα (-/-) mice fed with the 0.1% Wy-14,643 diet when compared with the controls.

3.5 Liver histology

To examine the liver damage resulting from Wy-14,643 treatment, liver sections from 24 hour, 1 week, 2 weeks, 6 months and 11 months Wy-14,643 treatment were used for histological studies. Figures 3.5.1 and 3.5.2 showed the central vein region of the four treatment groups after 24 hours gavage treatment with Wy-14,643 compound. Normal hepatocytes were arranged in a series of anastomosing plates with one cell thick. Between the anastomosing plates were sinusoidal spaces. These plates extended from the periphery of a lobule to the central vein at its center. Normal liver histology was found in the central vein (Figure s3.5.1A, 3.5.1B, 3.5.2A, 3.5.2B), middle zone (Figures 3.5.3A, 3.5.3B, 3.5.4A, 3.5.4B) and portal vein region (Figures 3.5.5A, 3.5.5B, 3.5.6A, 3.5.6B) of the PPARα (+/+) mice fed with a control or 0.1% Wy-14,643 diet. However, severe accumulation of lipid droplets was

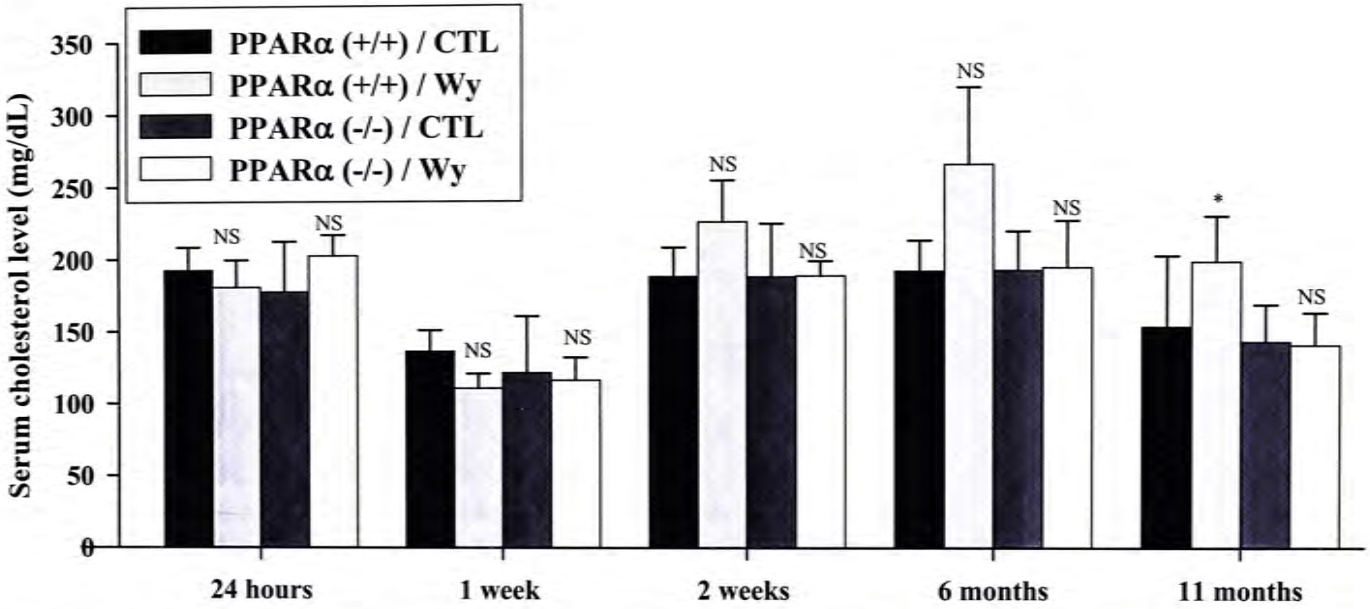

Figure 3.4.1. Effect of 24 hours, 1 week, 2 weeks, 6 months and 11 months 0.1% Wy-14643 treatment on serum cholesterol level of PPARα (+/+) and PPARα (-/-) mice. Data are expressed as mean ± SD (n ≥8). ANOVA on ranks was used to compare the differences in mean values within each time-point using SigmaStat Program. *Significant difference ($p \leq 0.05$) from control (CTL) fed PPARα (+/+) mice. NS, no significant difference.

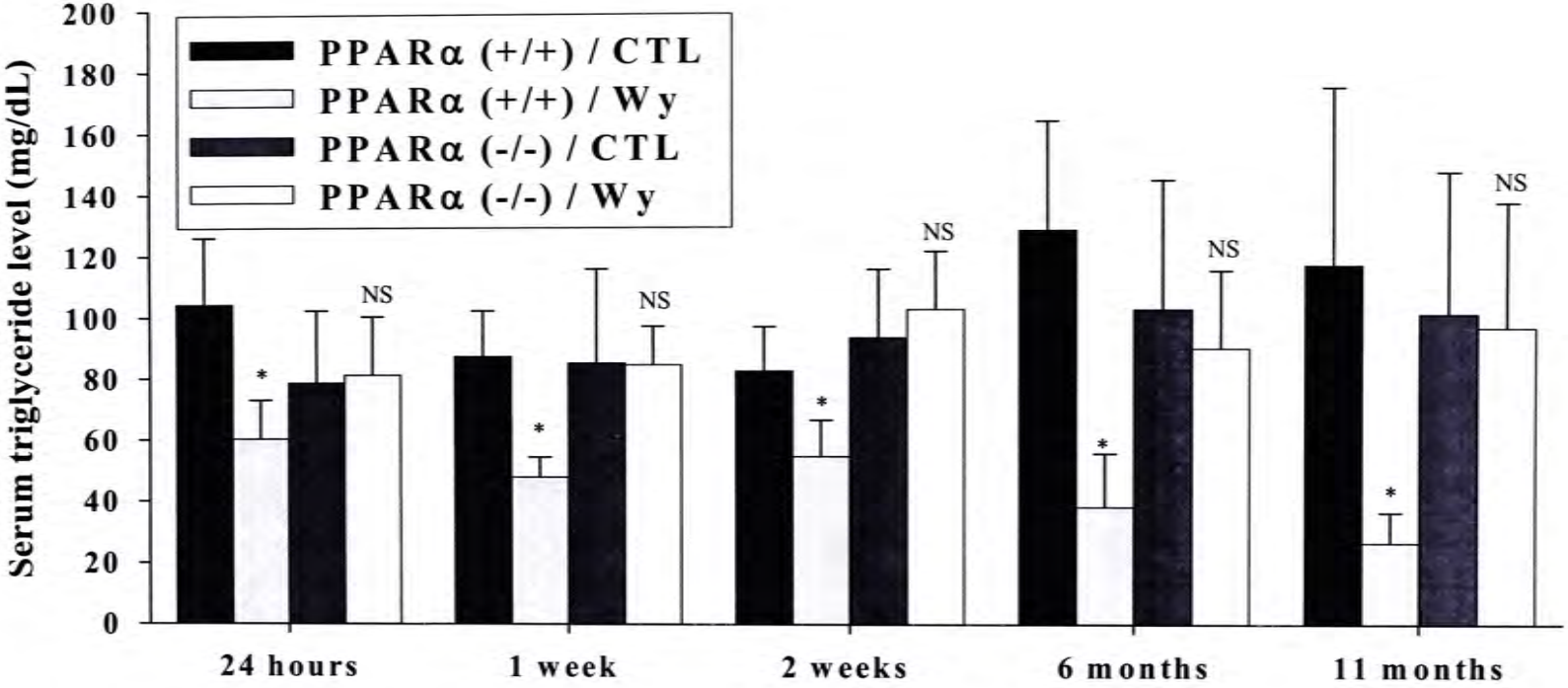

Figure 3.4.2. Effect of 24 hours, 1 week, 2 weeks, 6 months and 11 months 0.1% Wy-14643 treatment on serum triglyceride level of PPARα (+/+) and PPARα (-/-) mice. Data are expressed as mean ± SD (n ≥8). ANOVA on ranks was used to compare the differences in mean values within each time-point using SigmaStat Program. *Significant difference ($p \leq 0.05$) from control (CTL) fed PPARα (+/+).

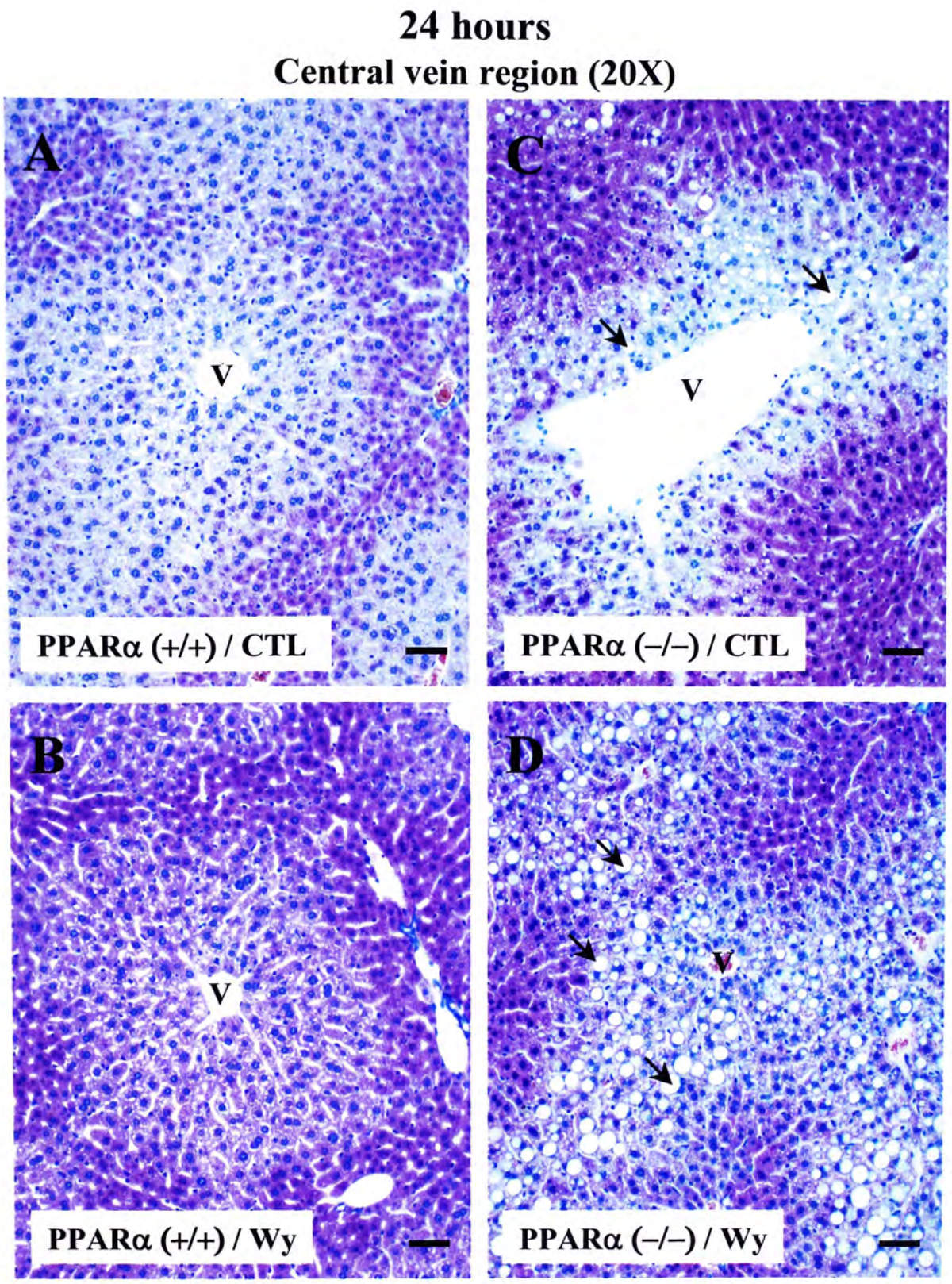

Figure 3.5.1. Hematoxylin and eosin staining of the central vein region (20X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 24 hours gavage feeding with 1% methylcellulose (control vehicle) (panels A, B) or Wy-14,643 (50 mg/kg bw) (panels C, D) (n=3). Hepatocytes were arranged radially from the central vein. Normal liver histology was found in the central vein region of both control vehicle and Wy-14,643 fed PPARα (+/+) mice. Severe lipid accumulation (arrows) was found around the central vein region in both control vehicle and Wy-14,643 fed PPARα (-/-) mice. V, central vein. Bars, 50 μm.

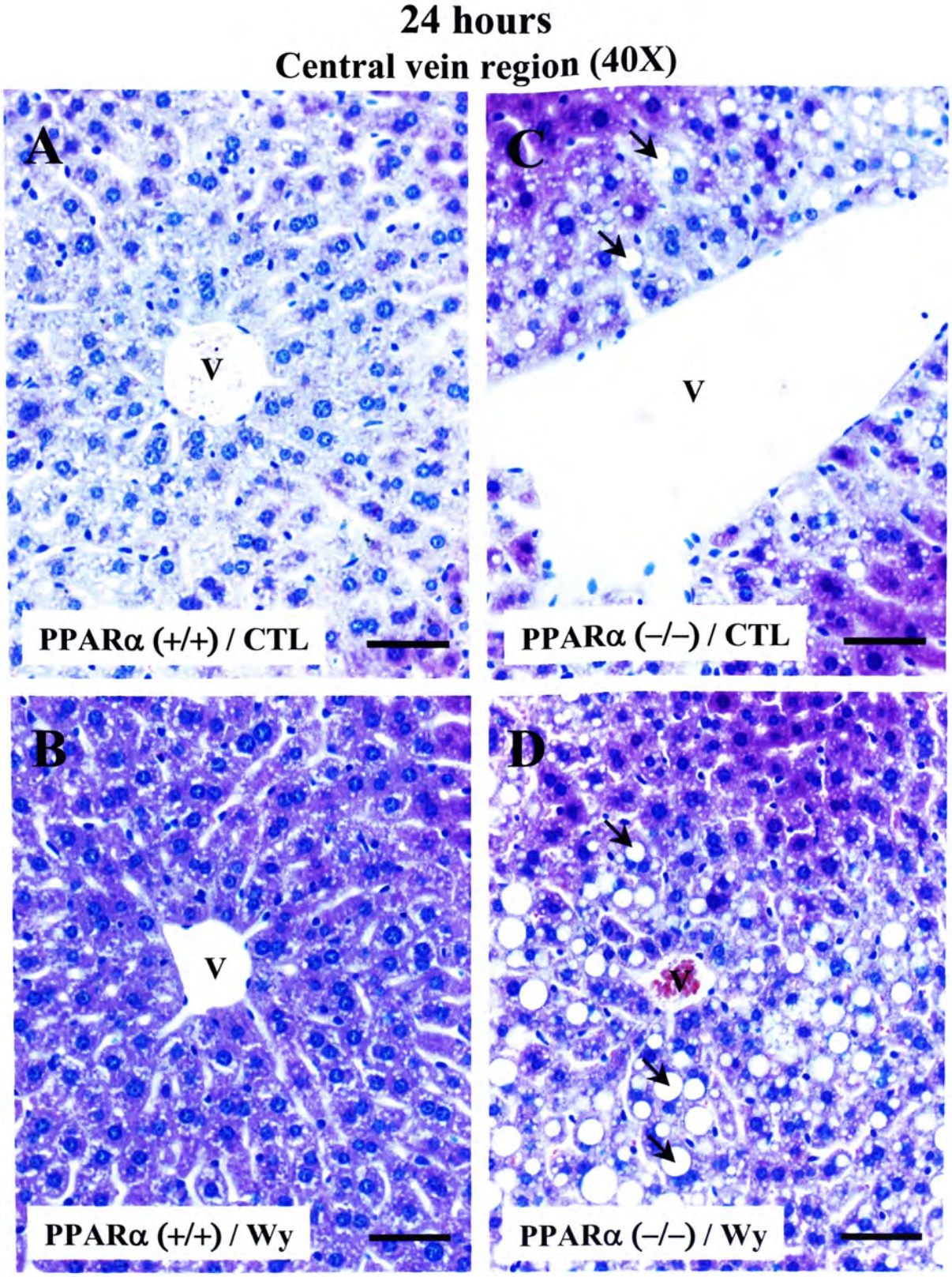

Figure 3.5.2. Hematoxylin and eosin staining of the central vein region (40X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 24 hours gavage feeding with 1% methylcellulose (control vehicle) (panels A, B) or Wy-14,643 (50 mg/kg bw) (panels C, D) (n=3). Normal liver histology was found in the central vein region of both control vehicle and Wy-14,643 fed PPARα (+/+) mice. Severe lipid accumulation (arrows) was found around the central vein region in both control vehicle and Wy-14,643 fed PPARα (-/-) mice. Macrovesicular droplets were formed inside the hepatocytes which displaced the nuclei to the periphery of the hepatocytes. V, central vein. Bars, 50 μm.

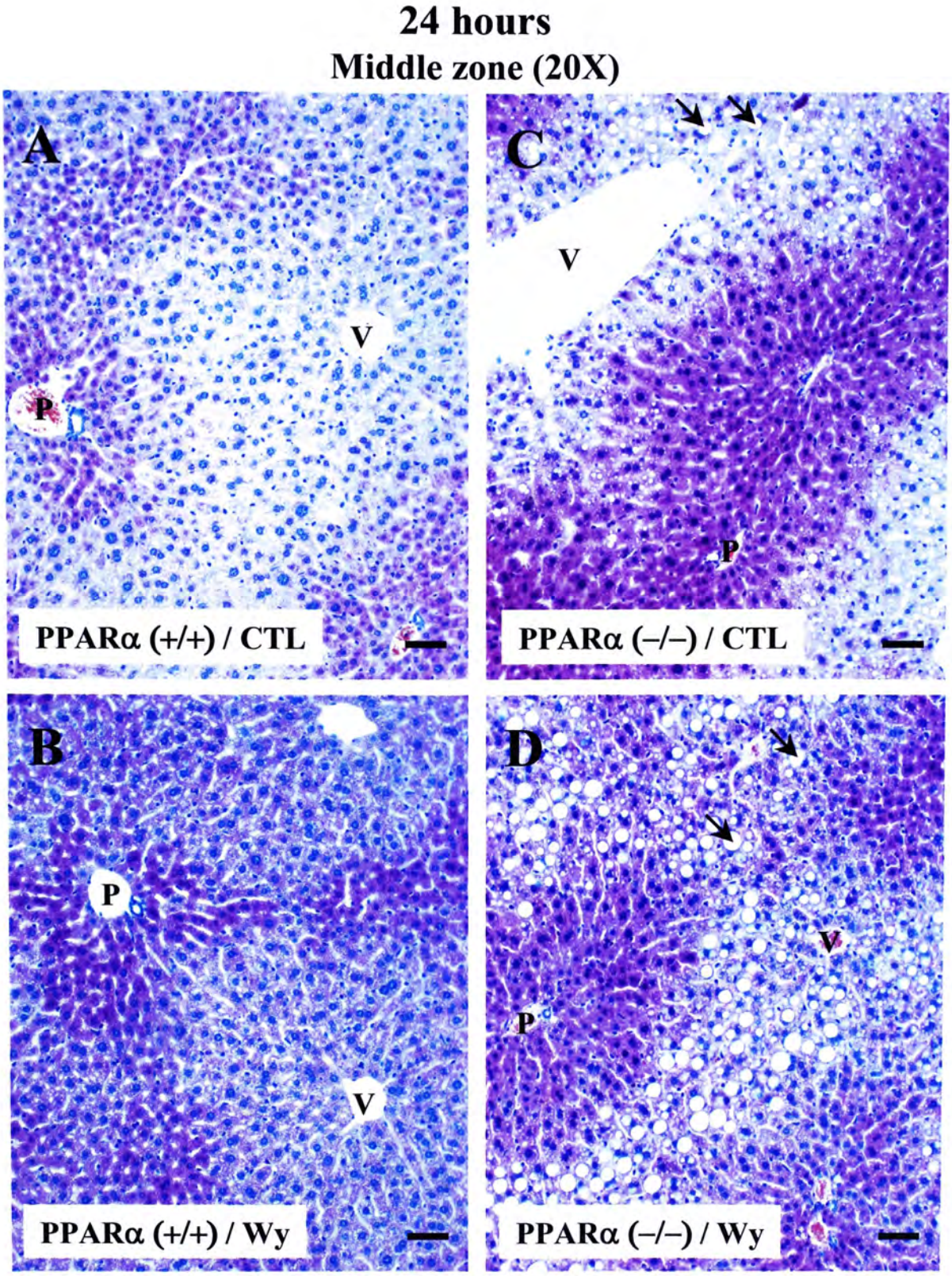

Figure 3.5.3. Hematoxylin and eosin staining of the middle zone (20X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 24 hours gavage feeding with 1% methylcellulose (control vehicle) (panels A, B) or Wy-14,643 (50 mg/kg bw) (panels C, D) (n=3). Normal liver histology was found in the middle zone of both control vehicle and Wy-14,643 fed PPARα (+/+) mice. Severe lipid accumulation (arrows) were found around the central vein region in both control vehicle and Wy-14,643 fed PPARα (-/-) mice. P, portal vein. V, central vein. Bars, 50 μm.

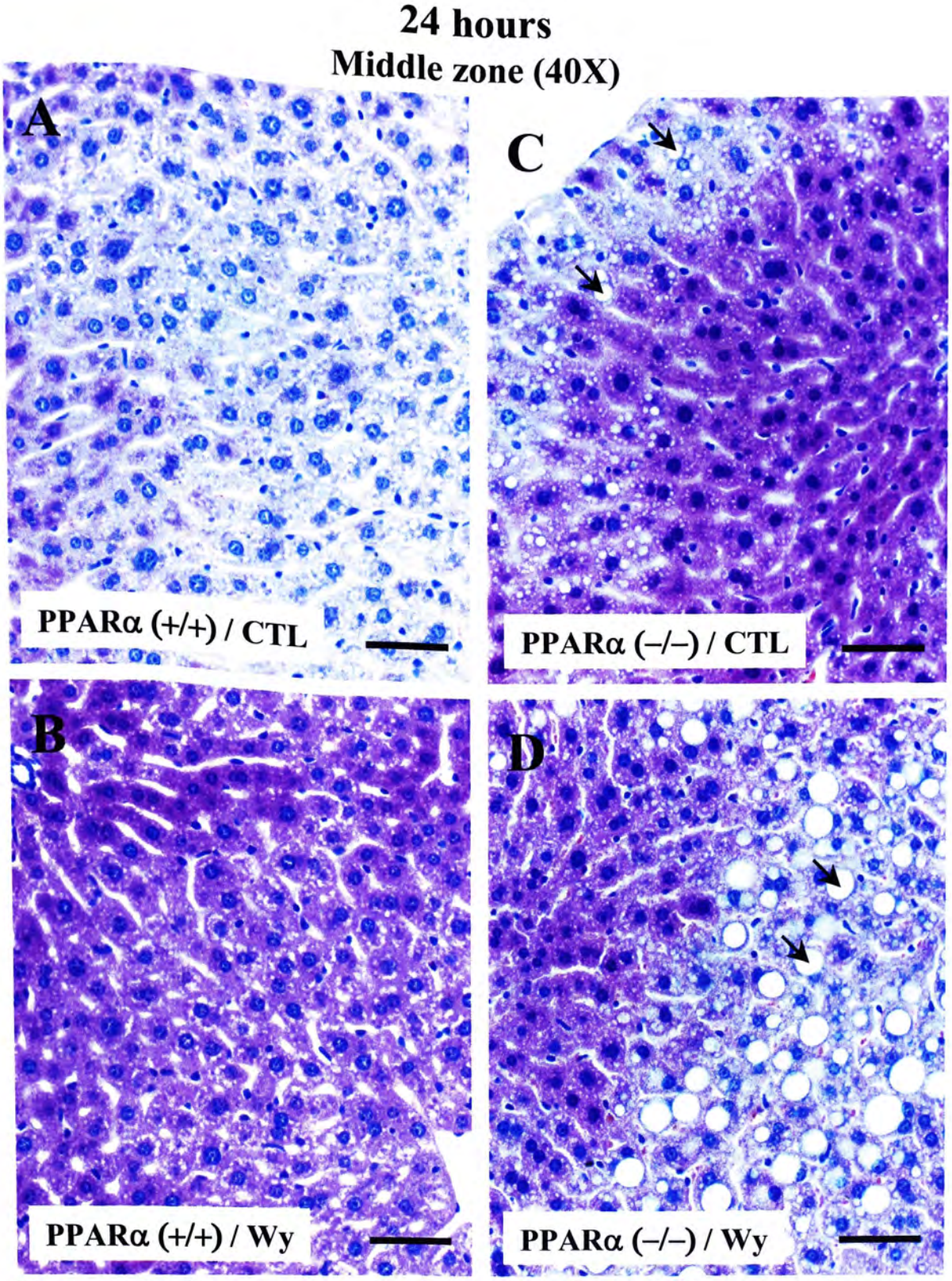

Figure 3.5.4. Hematoxylin and eosin staining of the middle zone (40X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 24 hours gavage feeding with 1% methylcellulose (control vehicle) (panels A, B) or Wy-14,643 (50 mg/kg bw) (panels C, D) (n=3). Normal liver histology was found in the middle zone of both control vehicle and Wy-14,643 fed PPARα (+/+) mice. P, portal triad. Bars, 50 μm.

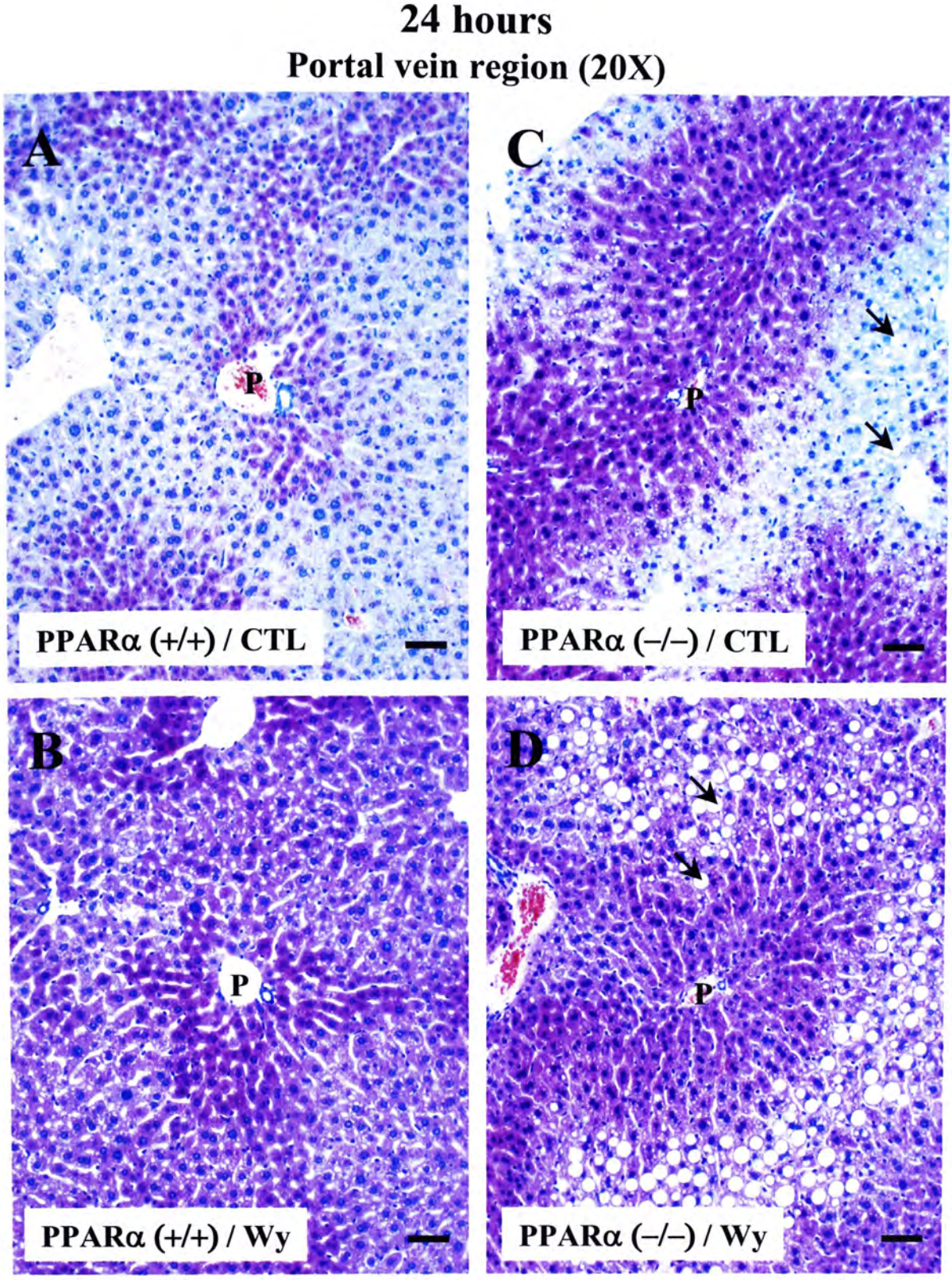

Figure 3.5.5. Hematoxylin and eosin staining of the portal vein region (20X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 24 hours gavage feeding with 1% methylcellulose (control vehicle) (panels A, B) or Wy-14,643 (50 mg/kg bw) (panels C, D) (n=3). Normal liver histology was found in the portal vein region of both control vehicle and Wy-14,643 fed PPARα (+/+) mice fed. P, portal triad. Bars, 50 μm.

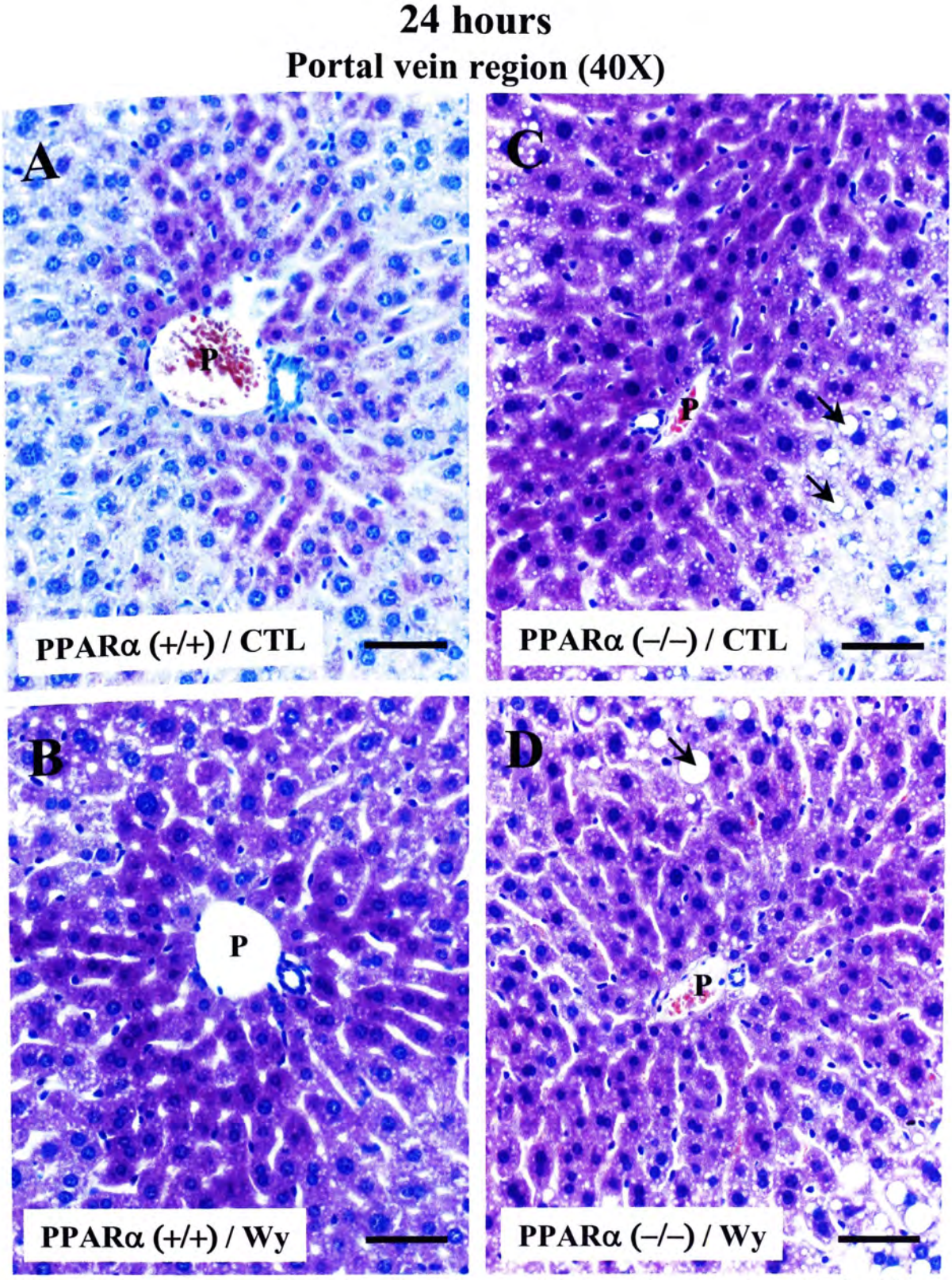

Figure 3.5.6. Hematoxylin and eosin staining of the portal vein region (40X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 24 hours gavage feeding with 1% methylcellulose (control vehicle) (panels A, B) or Wy-14,643 (50 mg/kg bw) (panels C, D) (n=3). Normal liver histology was found in the portal vein region of both control vehicle and Wy-14,543 fed PPARα (+/+) mice. P, portal triad. Bars, 50 μm.

observed near the central vein for both the control (Figure 3.5.1C) and 0.1% Wy-14,643 fed PPARα (-/-) mice (Figure 5.5.1D). Macrovesicular droplets were formed inside the hepatocytes which displaced the nuclei to the periphery of the hepatocytes (Figures 3.5.2C, 3.5.2D). The accumulation of the lipid droplets in both the control and treatment groups of the PPARα (-/-) mice suggesting that the disruption of PPARα gene is involved in the lipid metabolism.

Similar to the 24 hour gavage feeding, normal liver histology was found in the central vein (Figures 3.5.7A, 3.5.8A), middle zone (Figures 3.5.9A, 3.5.10A) and portal vein region (Figures 3.5.11A, 3.5.12A) in PPARα (+/+) mice fed with control diet. However, the normal arrangement of the hepatocytes was not found in the PPARα (+/+) mice fed with 0.1% Wy-14,643 (Figures 3.5.7B, 3.5.8B). The sinusoids between hepatocytes become larger in the 0.1% Wy-14,643-treated PPARα (+/+) mice. Accumulation of the lipid droplets were found around the central veins in both the control and treated groups of the PPARα (-/-) mice (Figures 3.5.7C, 3.5.7D, 3.5.8C, 3.5.8D).

Large sinusoidal spaces were observed in the PPARα (+/+) mice treated with 0.1% Wy-14,643 at 2 week feeding study in the central vein region (Figures 3.5.13B, 3.5.14B), middle zone (Figures 3.5.15B, 3.5.16B) and portal vein region (Figures 3.5.17B, 3.5.18B). In addition, abnormal and irregular hepatocytes arrangement was also found in the Wy-14,643-treated group in PPARα (+/+) mice in the central vein region (Figures 3.5.13B, 3.5.14B), middle zone (Figures 3.5.15B, 3.5.16B) and portal vein region (Figures 3.5.17B, 3.5.18B). Lipid accumulation was found in central vein region of both the control and treatment groups of the PPARα (-/-) mice (Figures 3.5.13C, 3.5.14D, 3.5.13C, 3.5.14D).

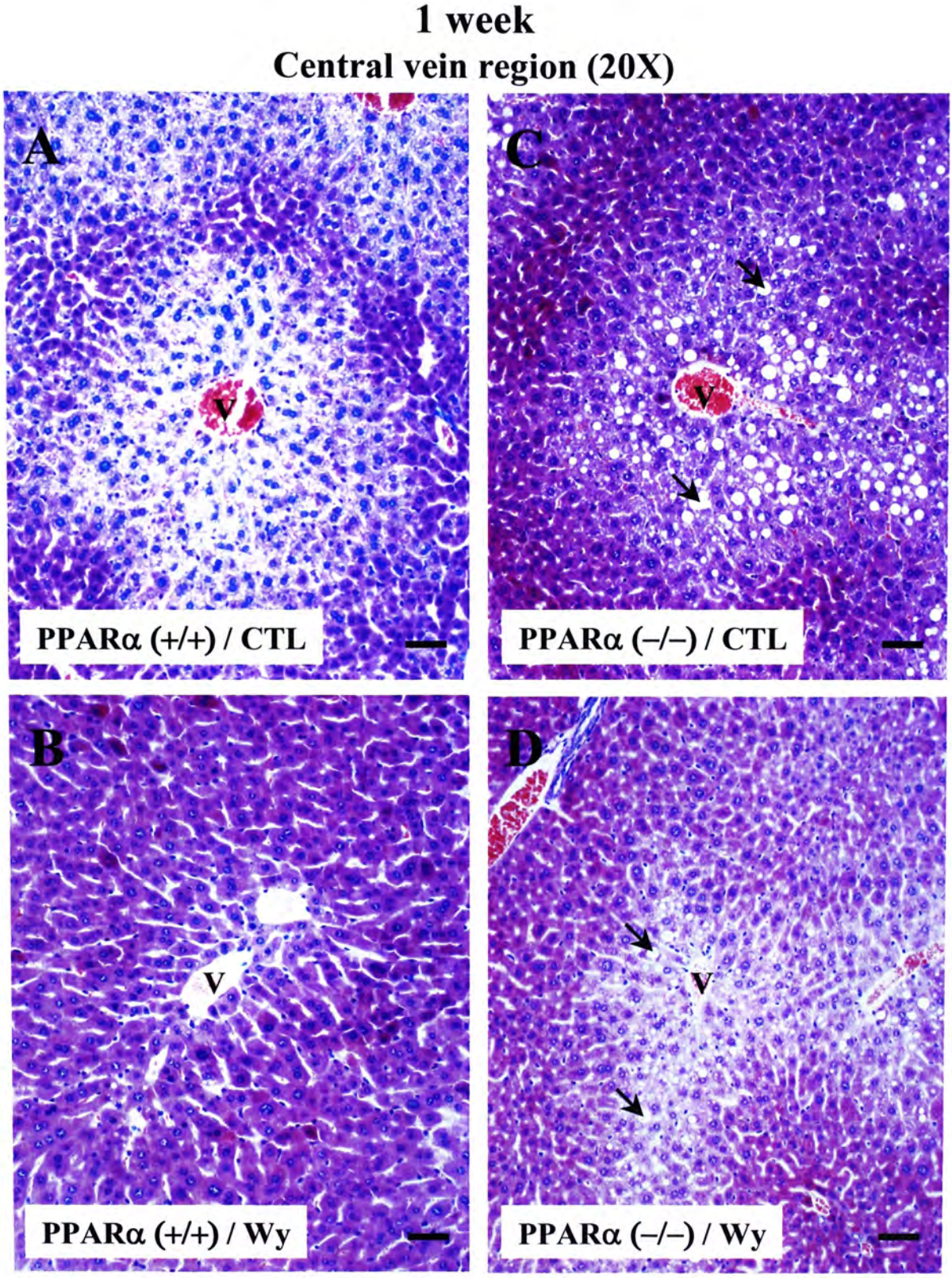

Figure 3.5.7. Hematoxylin and eosin staining of the central vein region (20X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 1 week treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Normal liver histology was found in central vein region of PPARα (+/+) mice fed with control diet. Increased sinusoid spaces was found in the 0.1% Wy-14,643 fed PPARα (+/+) mice. Severe lipid accumulation (arrows) was found around the central vein region in both control and Wy-14,643 fed PPARα (-/-) mice. V, central vein. Bars, 50 μm.

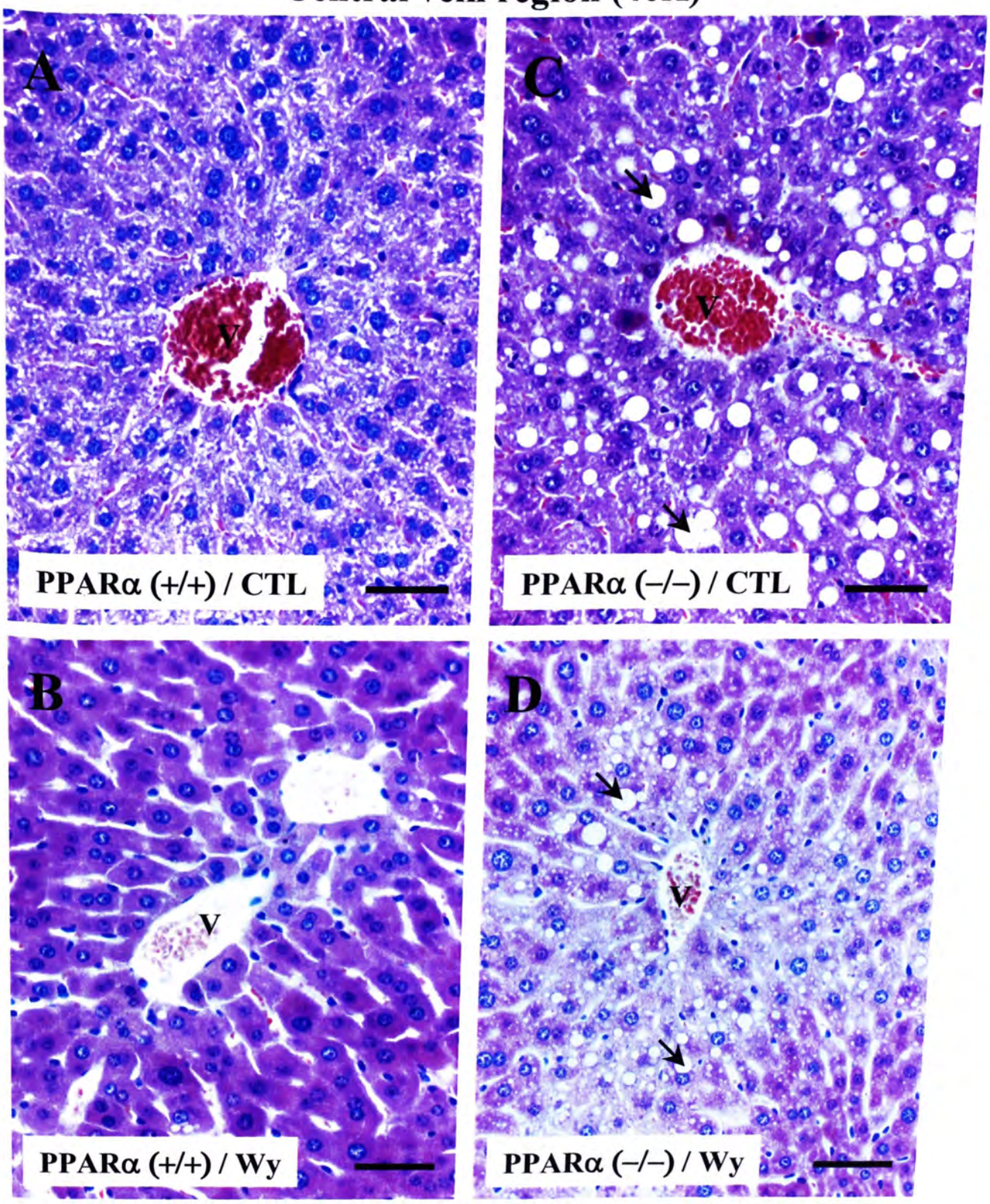

Figure 3.5.8. Hematoxylin and eosin staining of the central vein region (40X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 1 week treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Sinusoidal spaces between hepatocytes of PPARα (+/+) 0.1% Wy-14,643 treated mice were more obvious. Severe lipid accumulation (arrows) was found around the central vein region in both control and Wy-14,643 diet fed PPARα (-/-) mice. Macrovesicular droplets were formed inside the hepatocytes and displace the nuclei to the periphery of the hepatocytes. V, central vein. Bars, 50 μm.

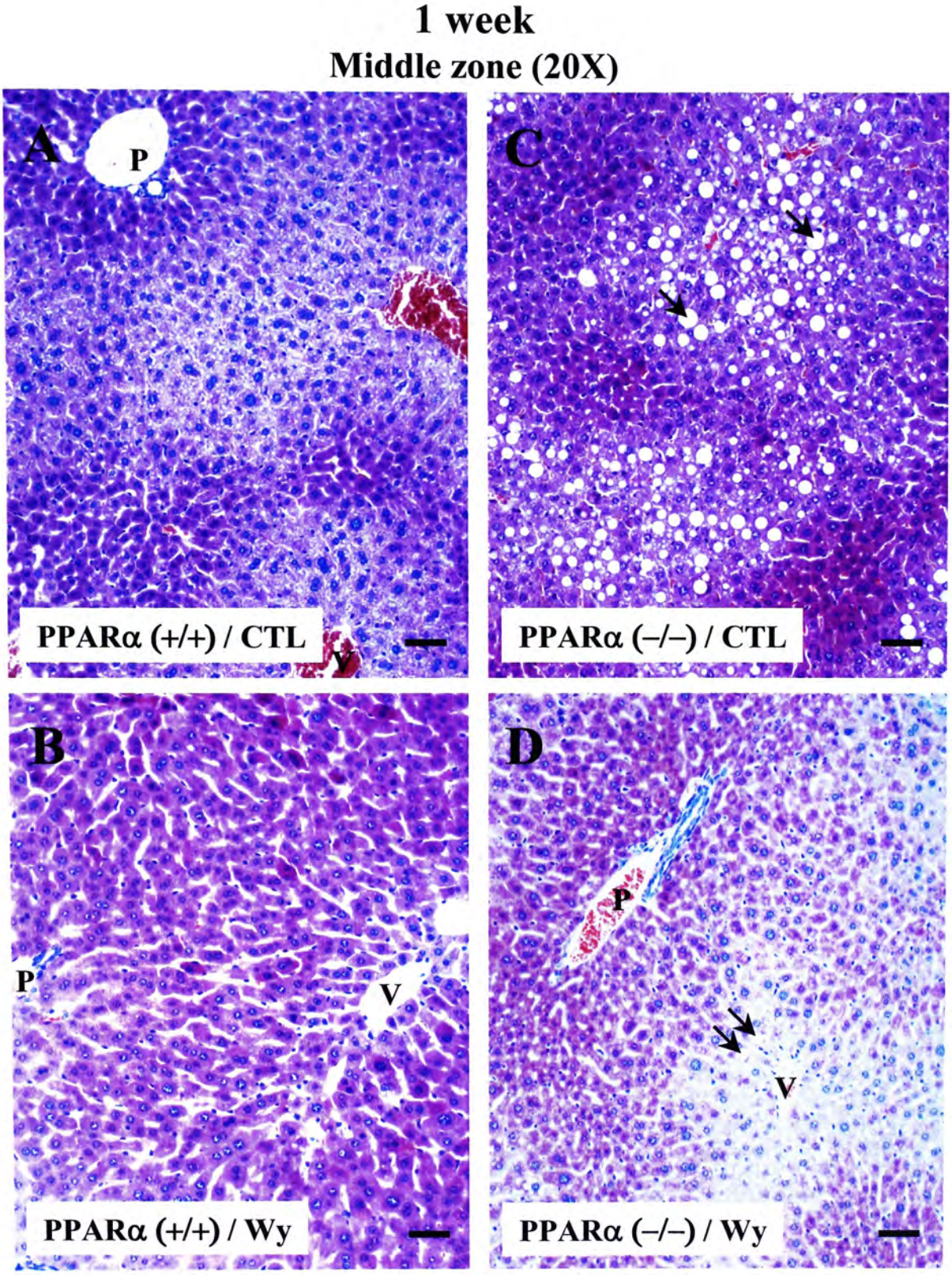

Figure 3.5.9. Hematoxylin and eosin staining of the middle zone (20X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 1 week treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Severe lipid accumulation (arrows) was found around the central vein region in both control and Wy-14,643 fed PPARα (-/-) mice. P, portal triad. V, central vein. Bars, 50 μm.

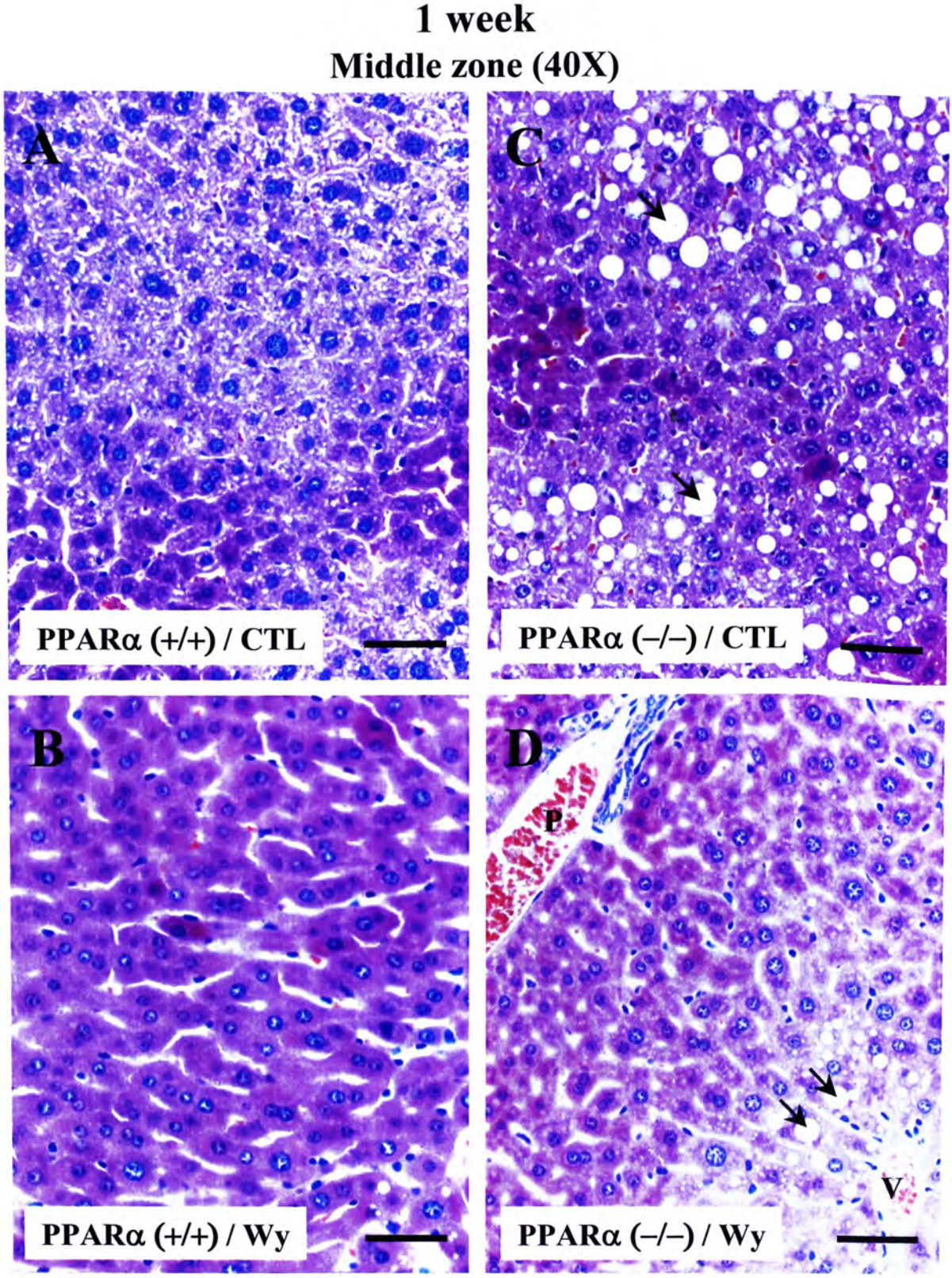

Figure 3.5.10. Hematoxylin and eosin staining of the middle zone (40X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 1 week treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Lipid accumulation (arrows) was found around the central vein region in both control and Wy-14,643 fed PPARα (-/-) mice. P, portal triad. V, central vein. Bars, 50 μm.

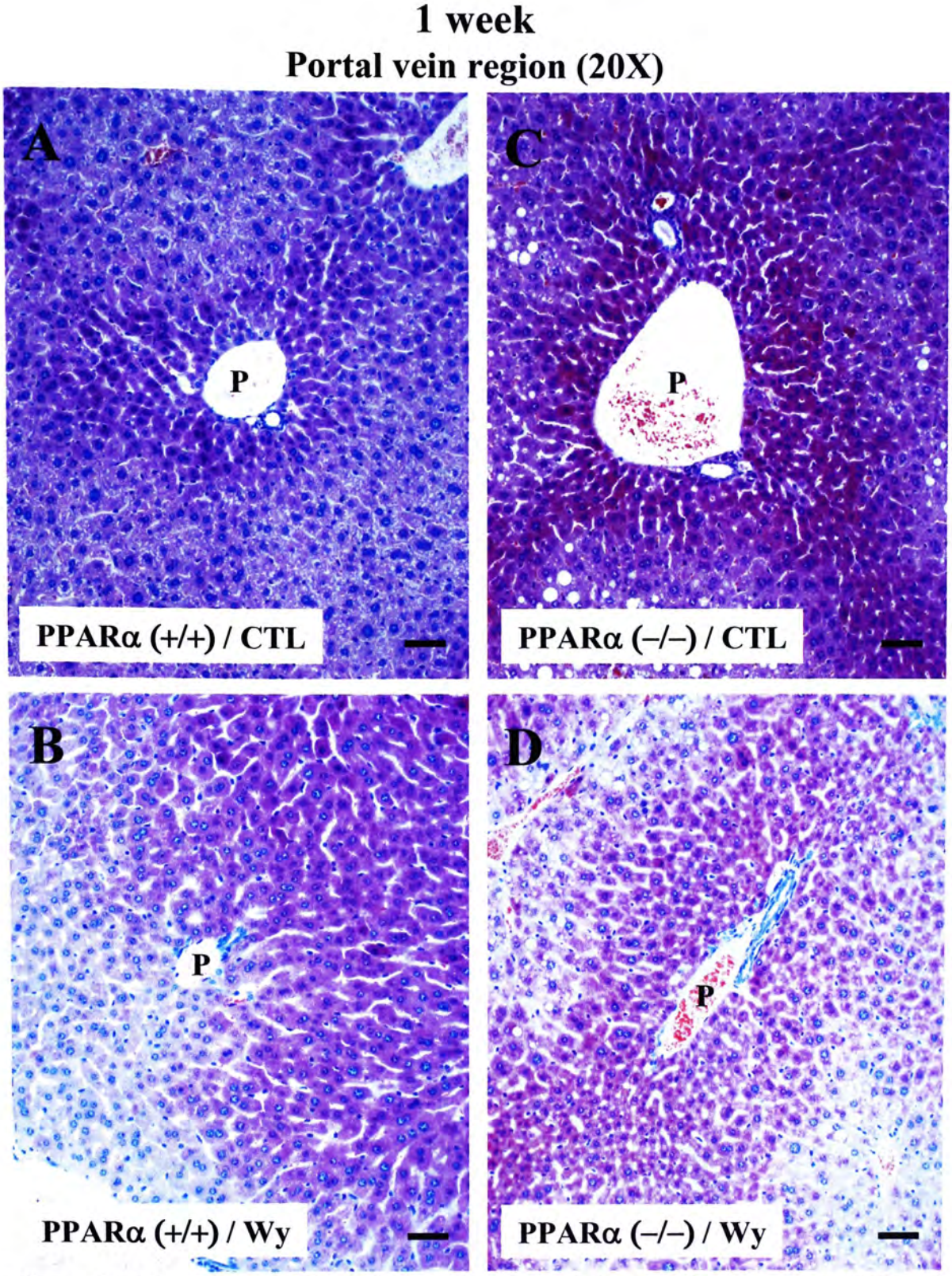

Figure 3.5.11. Hematoxylin and eosin staining of the portal vein region (20X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 1 week treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Normal liver histology was found in the portal vein region of control fed PPARα (+/+) mice. Sinusoidal spaces between hepatocytes of PPARα (+/+) 0.1% Wy-14,643 treated mice were more obvious. P, portal triad. Bars, 50 μm.

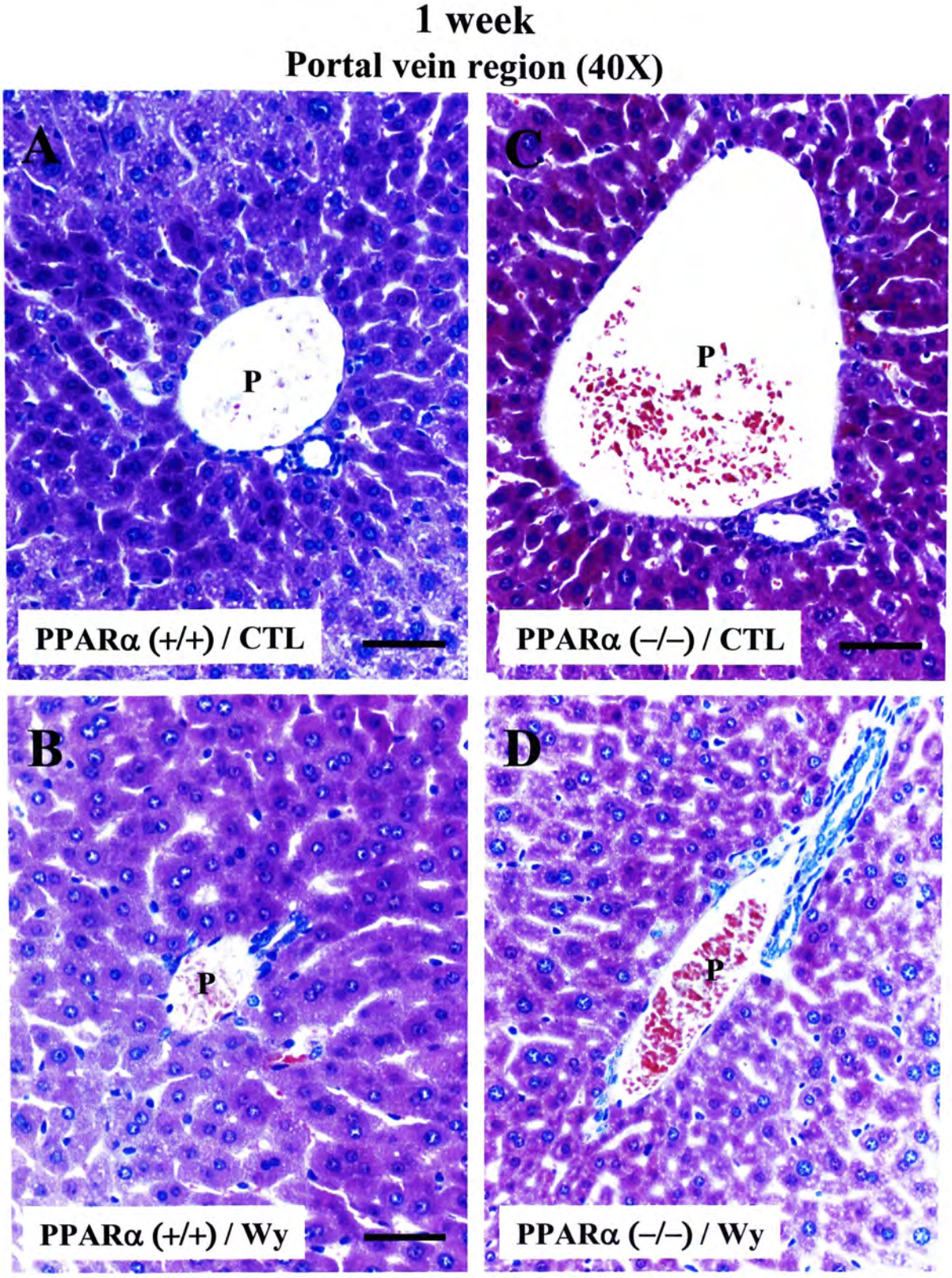

Figure 3.5.12. Hematoxylin and eosin staining of the portal vein region (40X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 1 week treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Normal liver histology was found in the portal vein region of control fed PPARα (+/+) mice. Sinusoidal spaces between hepatocytes of PPARα (+/+) 0.1% Wy-14,643 treated mice were more obvious. P, portal triad. Bars, 50 μm.

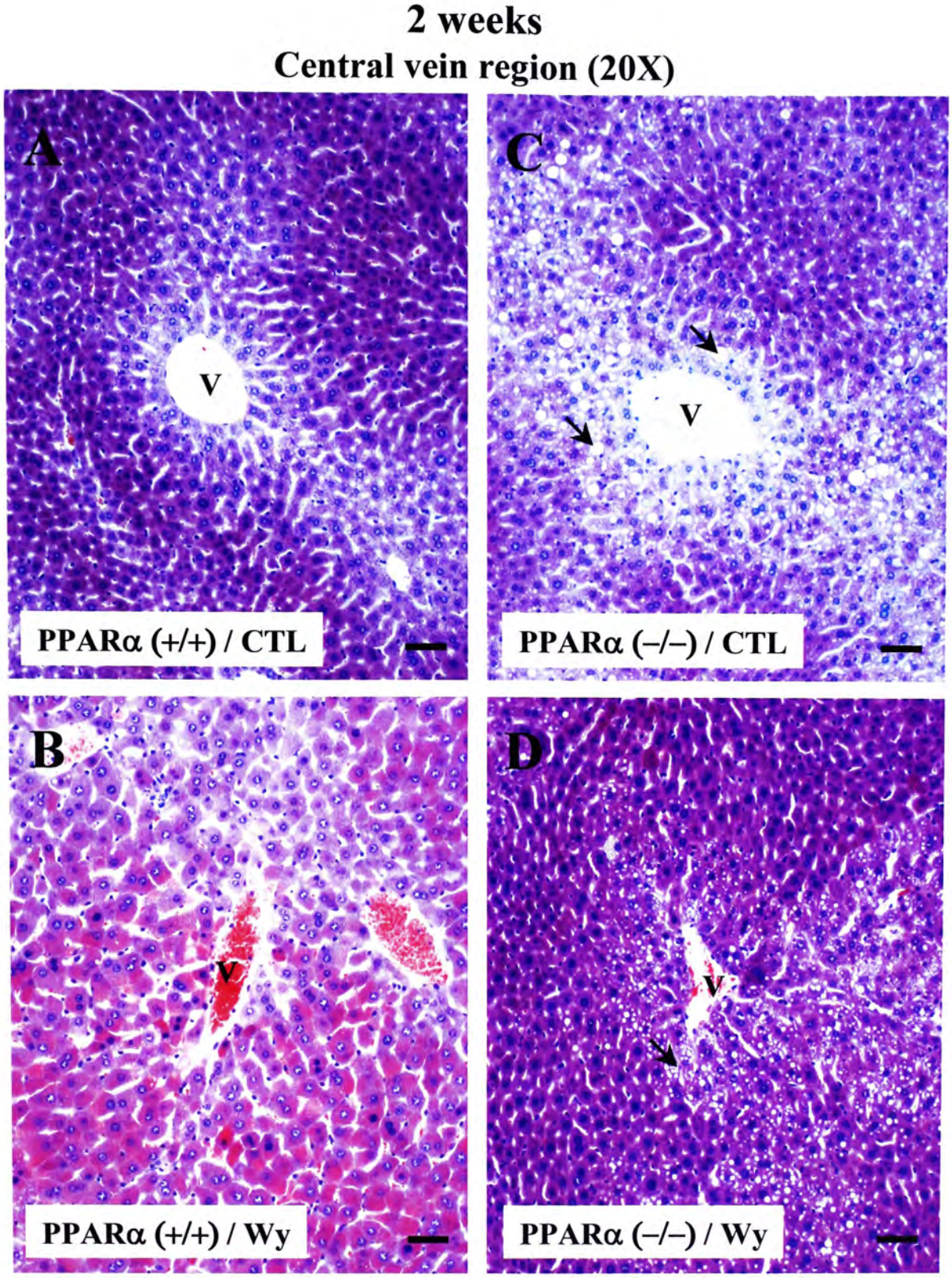

Figure 3.5.13. Hematoxylin and eosin staining of the central vein region (20X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 2 weeks treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Normal liver histology was found in the central vein region of PPARα (+/+) mice fed with control diet. Larger sinusoidal spaces were observed in the 0.1% Wy-14,643 fed PPARα (+/+) mice. Severe lipid accumulation (arrows) was found around the central vein region in both control and Wy-14,643 fed PPARα (-/-) mice. V, central vein. Bars, 50 μm.

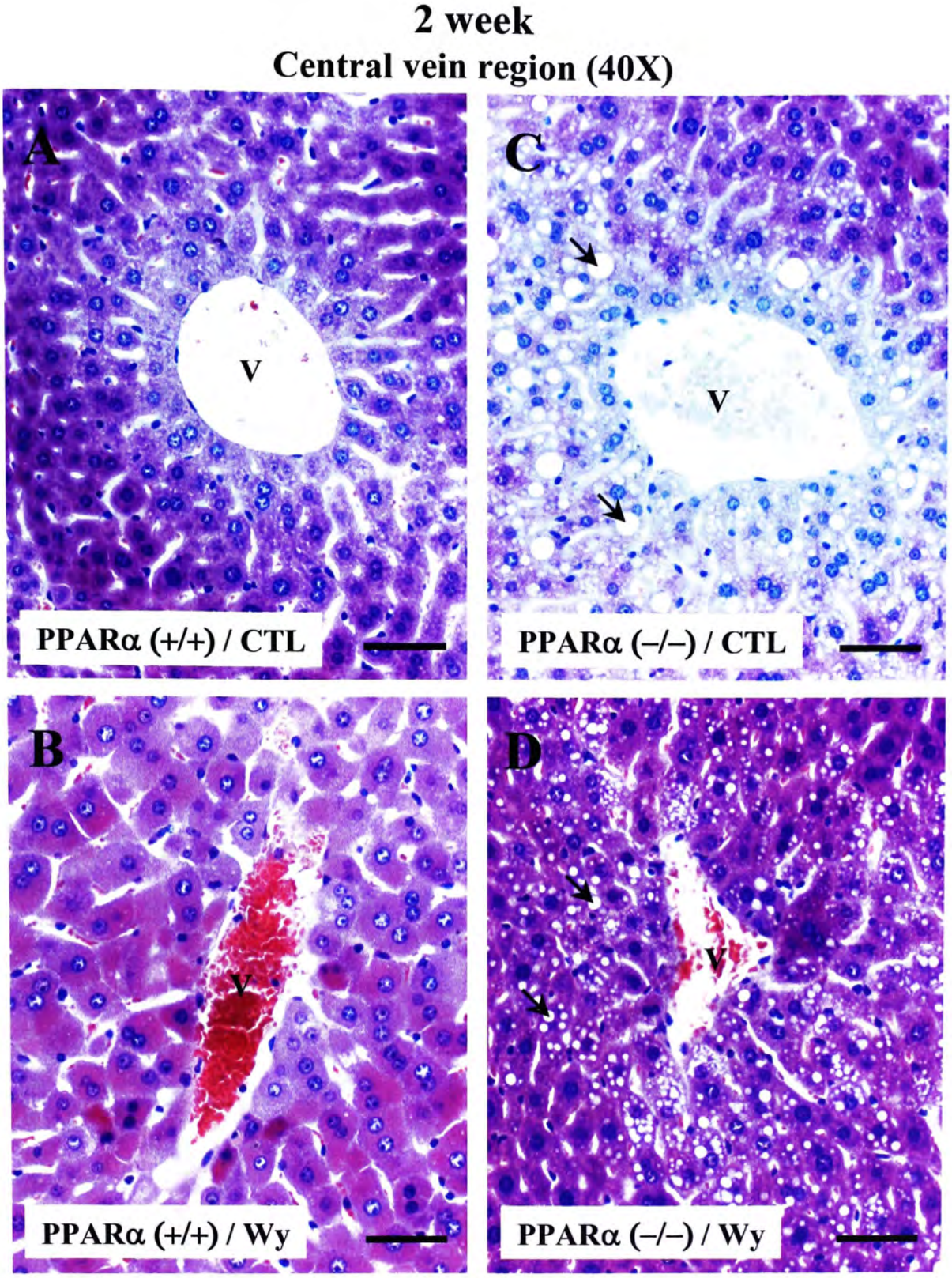

Figure 3.5.14. Hematoxylin and eosin staining of the central vein region (40X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 2 weeks treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Severe lipid accumulation (arrows) was found around the central vein region in both control and Wy-14,643 fed PPARα (-/-) mice. Macrovesicular droplets were formed inside the hepatocytes. The nuclei were displaced to the periphery of the hepatocytes. V, central vein. Bars, 50 μm.

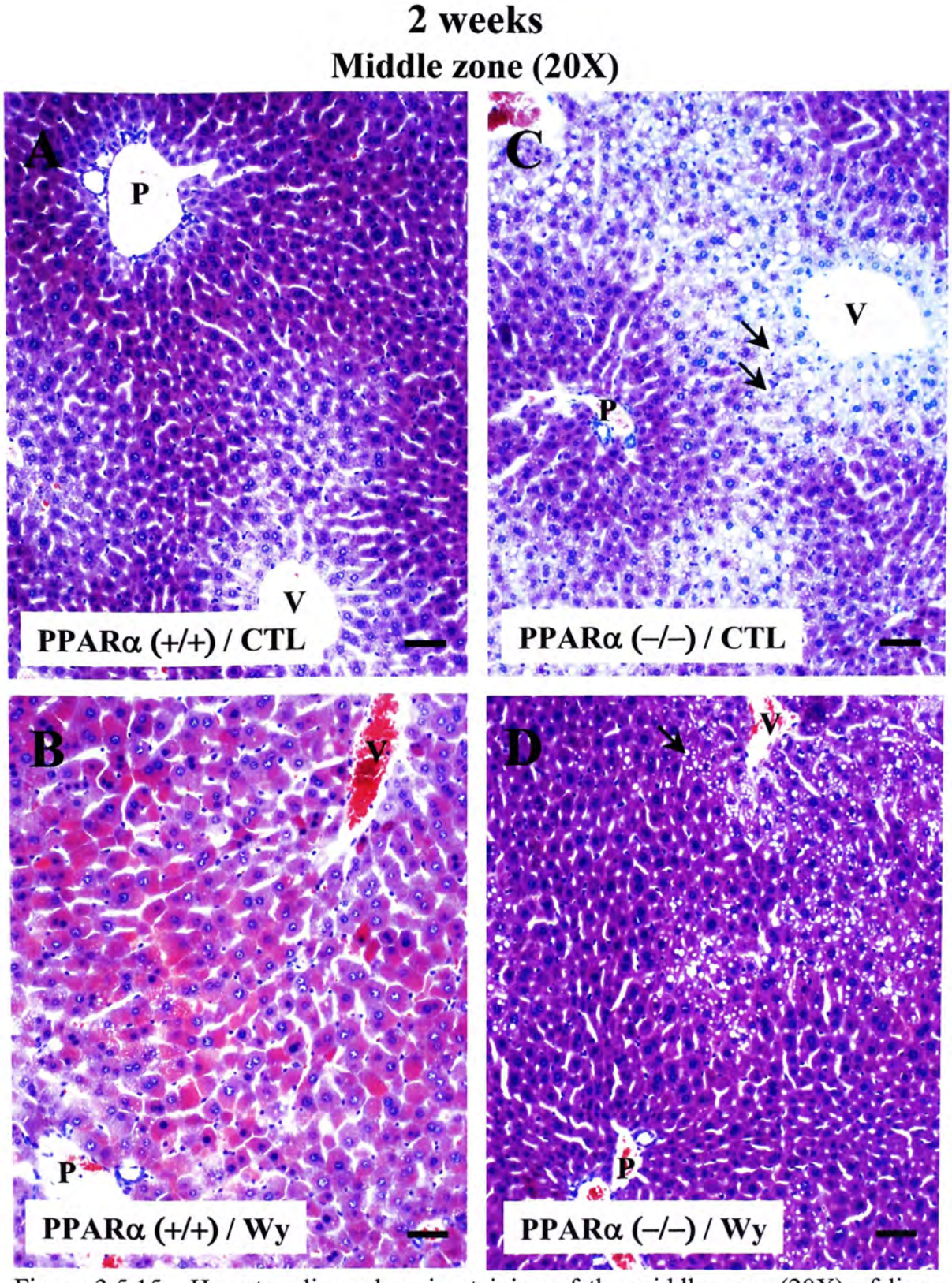

Figure 3.5.15. Hematoxylin and eosin staining of the middle zone (20X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 2 weeks treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Normal liver histology was found in the middle zone region of PPARα (+/+) mice fed with control diet. Larger sinusoidal spaces were observed in the 0.1% Wy-14,643 fed PPARα (+/+) mice. Severe lipid accumulation (arrows) was found around the central vein region in both control and Wy-14,643 fed PPARα (-/-) mice. P, portal triad. V, central vein. Bars, 50 μm.

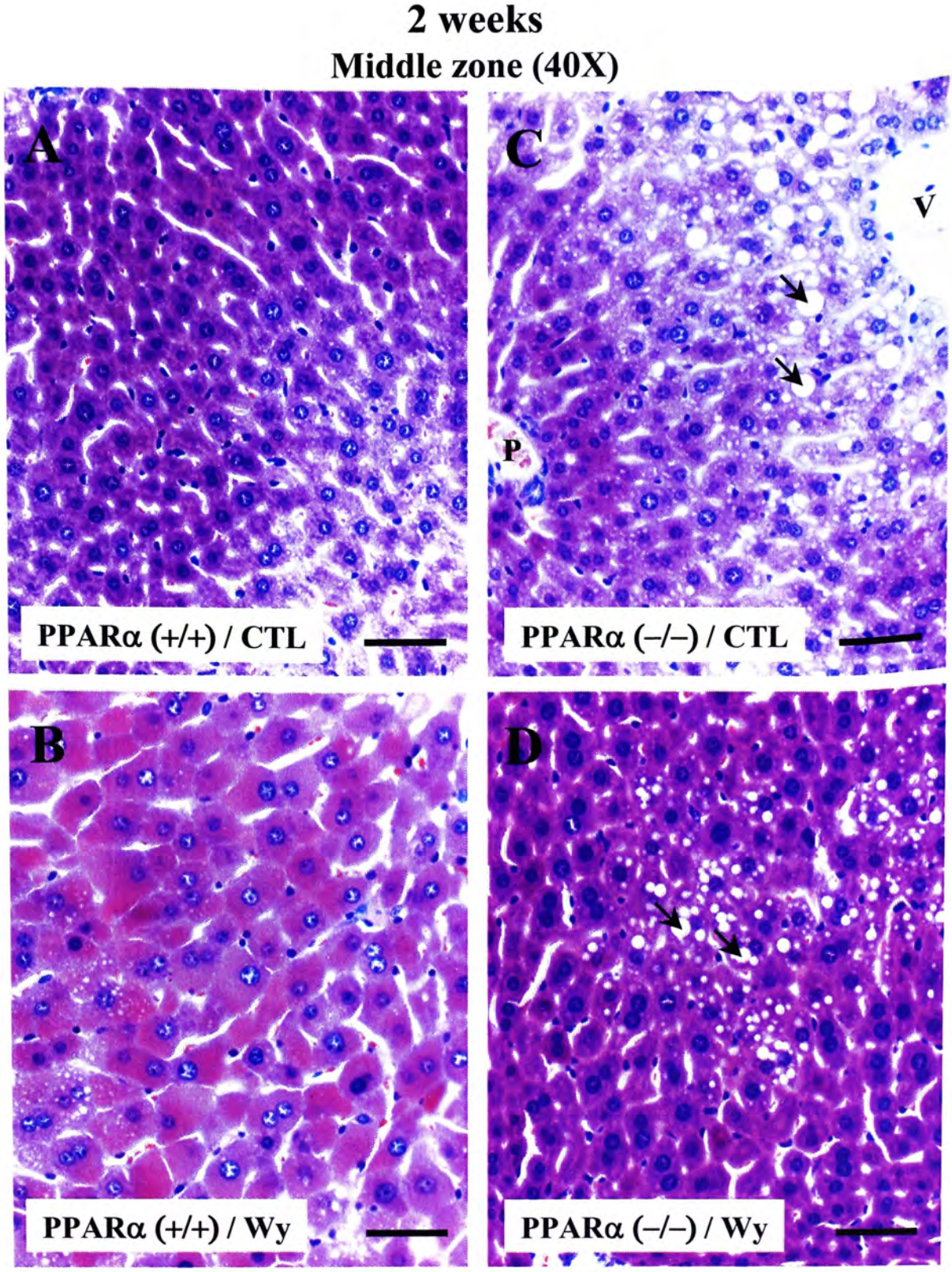

Figure 3.5.16. Hematoxylin and eosin staining of the middle zone (40X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 2 weeks treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Lipid accumulation (arrows) was found in both control and Wy-14,643 fed PPARα (-/-) mice. P, portal triad. V, central vein. Bars, 50 μm.

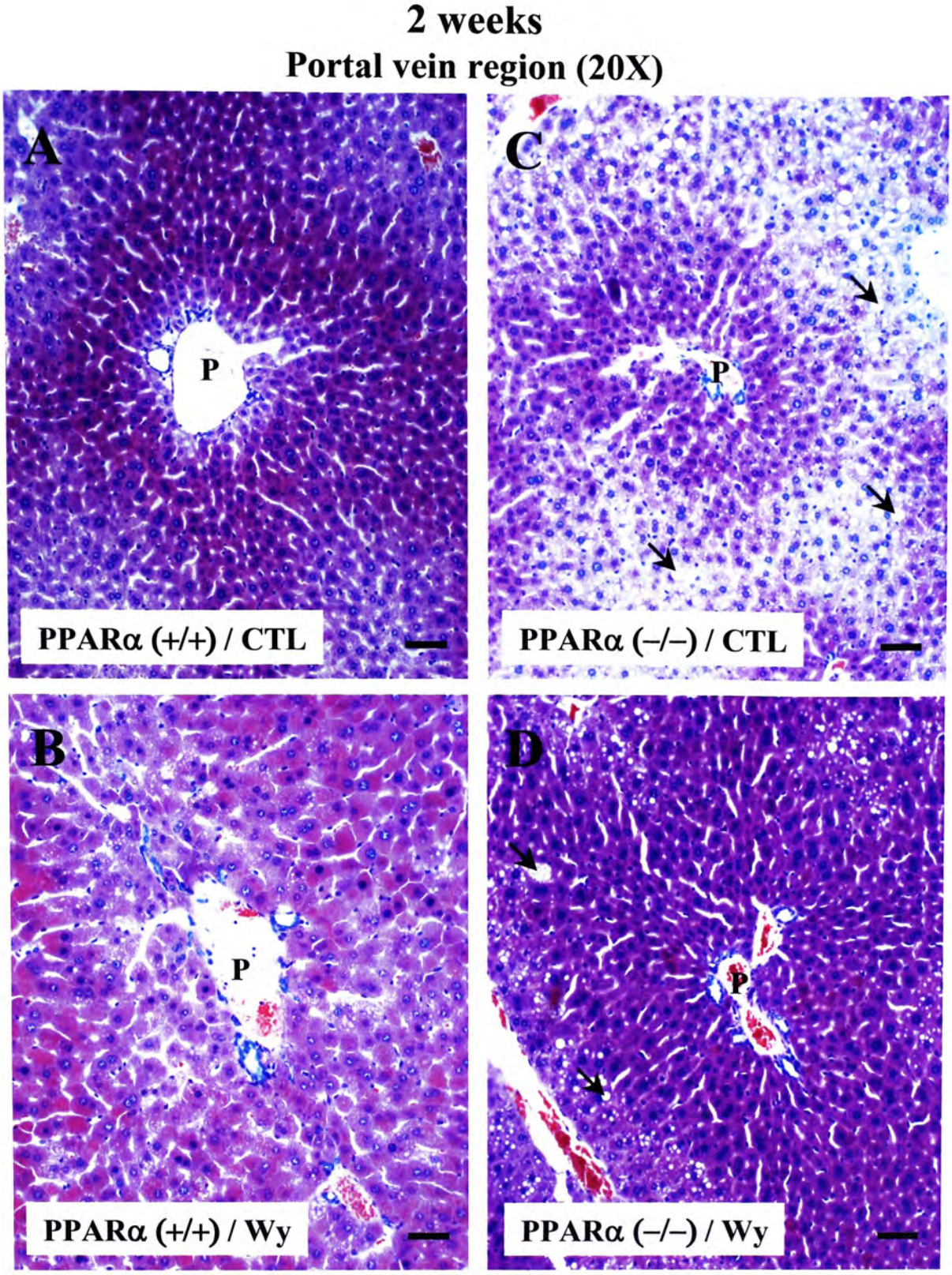

Figure 3.5.17. Hematoxylin and eosin staining of the portal vein region (20X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 2 weeks treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Normal liver histology was found in the portal vein region of control fed PPARα (+/+) mice. Abnormal arrangement of hepatocytes in the 0.1% Wy-14,643 treated PPARα (+/+) was observed. P, portal triad. Bars, 50 μm.

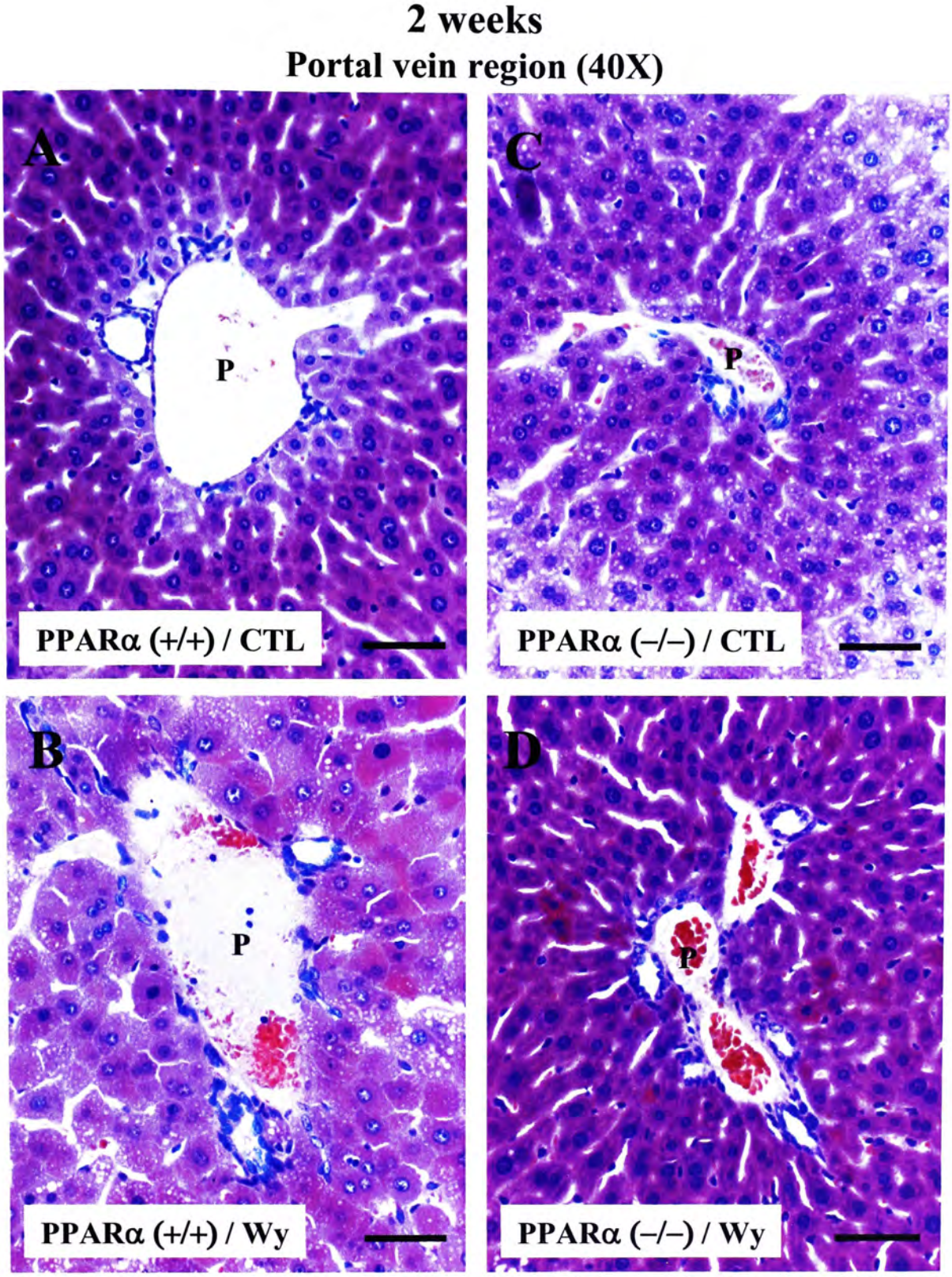

Figure 3.5.18. Hematoxylin and eosin staining of the portal vein region (40X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 2 weeks treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Normal liver histology was found in the portal vein region of control fed PPARα (+/+) mice. Abnormal arrangement of hepatocytes in the 0.1% Wy-14,643 treated PPARα (+/+) was observed. P, portal triad. Bars, 50 μm.

Abnormal hepatocytes arrangement and large sinusoids were more obvious in the 6 months feeding study. Trabecular cell clusters were observed in PPARα (+/+) mice fed with the 0.1% Wy-14,643 diet in the central vein region (Figures 3.5.19B, 3.5.20B), middle zone (Figure 3.5.21B, 3.5.22B) and portal vein region (Figures 3.5.23B, 3.5.24B). In addition, lipid droplets were accumulated near the portal vein region of the PPARα (+/+) mice fed with the 0.1% Wy-14,643 diet (Figure 3.5.22B, 3.5.24B). For PPARα (-/-) mice, both control and Wy-14,643-treated groups showed accumulation of the lipid droplets around the central vein (Figures 3.5.19C, 3.5.19D, 3.5.20C, 3.5.20D).

After 11 months Wy-14,643 treatment, abnormal hepatocytes arrangement, large sinusoids in the central vein region (Figures 3.5.25B, 2.5.26B), middle zone (Figures 3.5.27B, 3.5.28B) and portal vein region (Figure 3.5.29B, 3.5.30B) and lipid accumulation (Figures 3.5.29B, 3.5.30B) around the portal vein region were more obvious in the Wy-14,643 treated group of PPARα (+/+) mice. The accumulation of lipid droplets around the central vein for the PPARα (-/-) mice (Figures 3.5.25C, 3.5.25D) was very severe as the lipid metabolism was affected with the removal of peroxisome proliferators-activated receptor alpha. Irregular and trabecular cell clusters were found in the 0.1% Wy-14,643 fed PPARα (+/+) mice (Figures 3.5.25B, 3.5.27B, 3.5.29B).

Hepatocytes without nuclei (Figures 3.5.31A, 3.5.31D), trabecular cell clusters (Figure 3.5.31B, 3.5.31E) and invasion of blood, dark nucleus (Figures 3.5.31C, 3.5.31F) were observed in Wy-14,643-treated PPARα (+/+) after 11 months treatment. Trabecular cell clusters observed in 0.1% Wy-14,643 fed PPARα (+/+) mice was reported in previous study (Peters *et al.*, 1997).

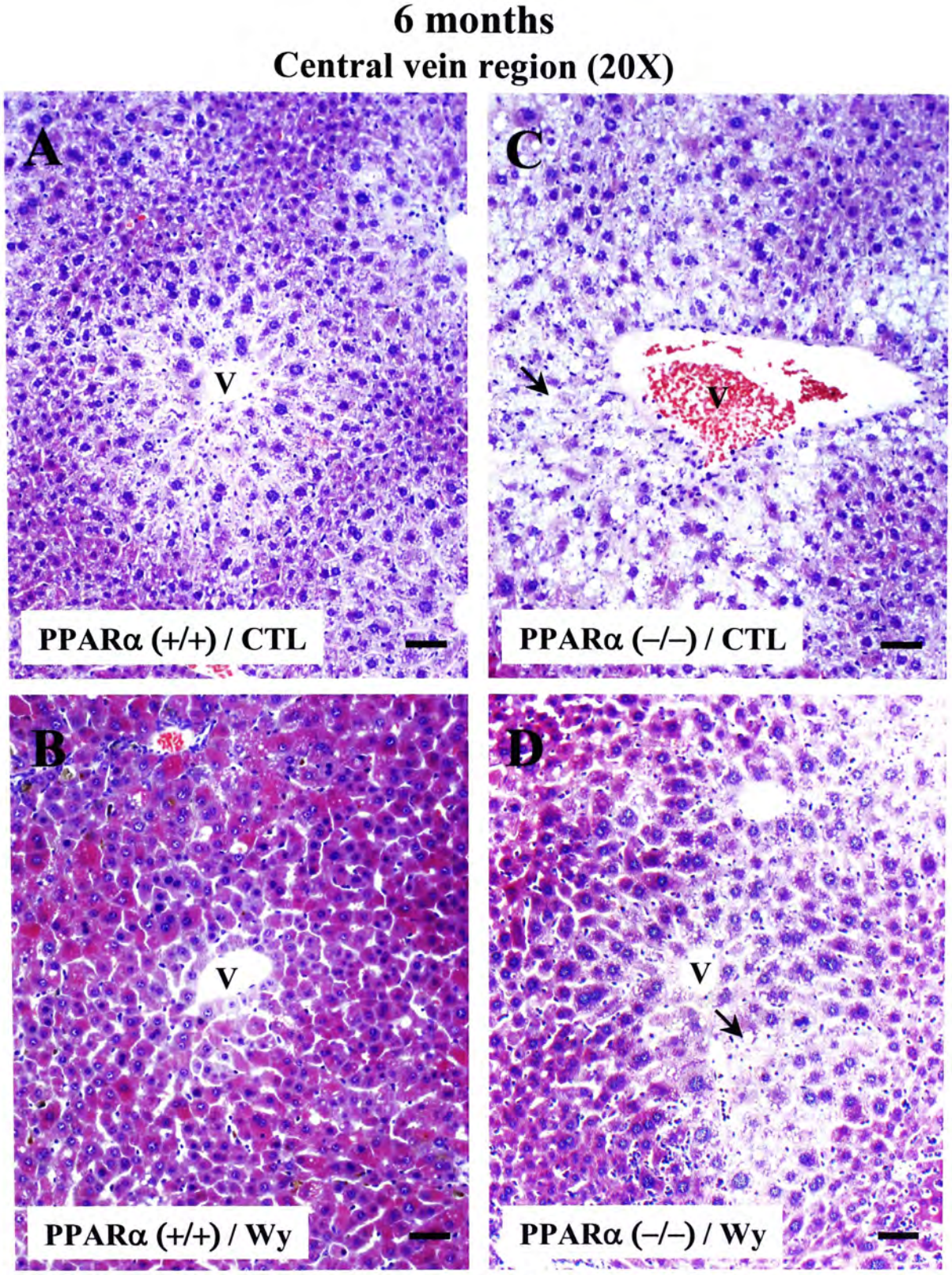

Figure 3.5.19. Hematoxylin and eosin staining of the central vein region (20X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 6 months treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Normal liver histology was found in the central vein region of PPARα (+/+) mice fed with control diet. Severe lipid accumulation (arrows) was found around the central vein region in both control and Wy-14,643 fed PPARα (-/-) mice. Irregular and trabecular cell clusters were observed in Wy-14,643 treated PPARα (+/+) mice. V, central vein. Bars, 50 μm.

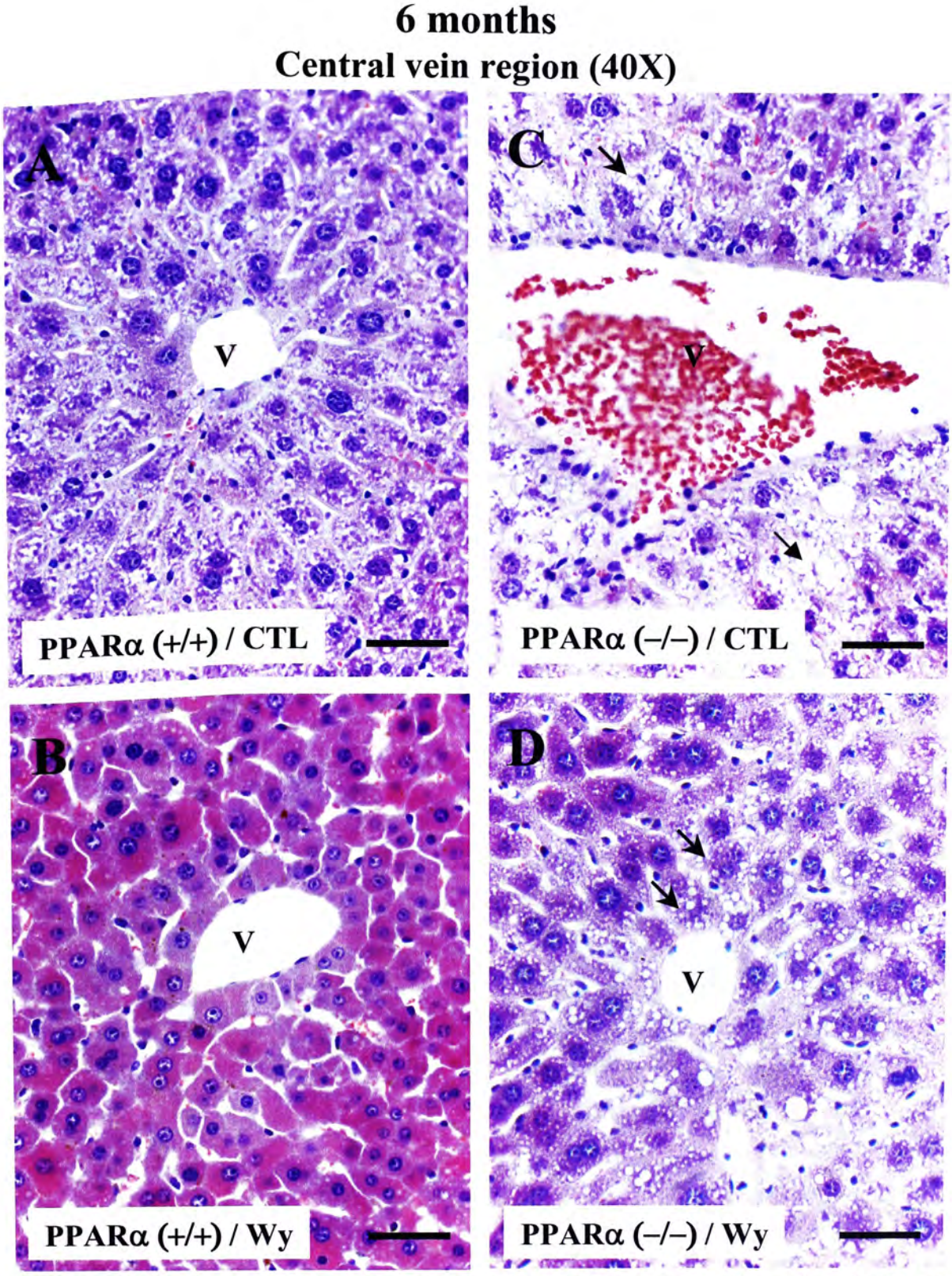

Figure 3.5.20. Hematoxylin and eosin staining of the central vein region (40X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 6 months treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Normal liver histology was found in the central vein region of PPARα (+/+) mice fed with control diet. Severe lipid accumulation (arrows) was found around the central vein region in both control and Wy-14,643 fed PPARα (-/-) mice. Macrovesicular droplets were formed inside the hepatocytes and the nuclei were displaced to the periphery of the hepatocytes. V, central vein. Bars, 50 μm.

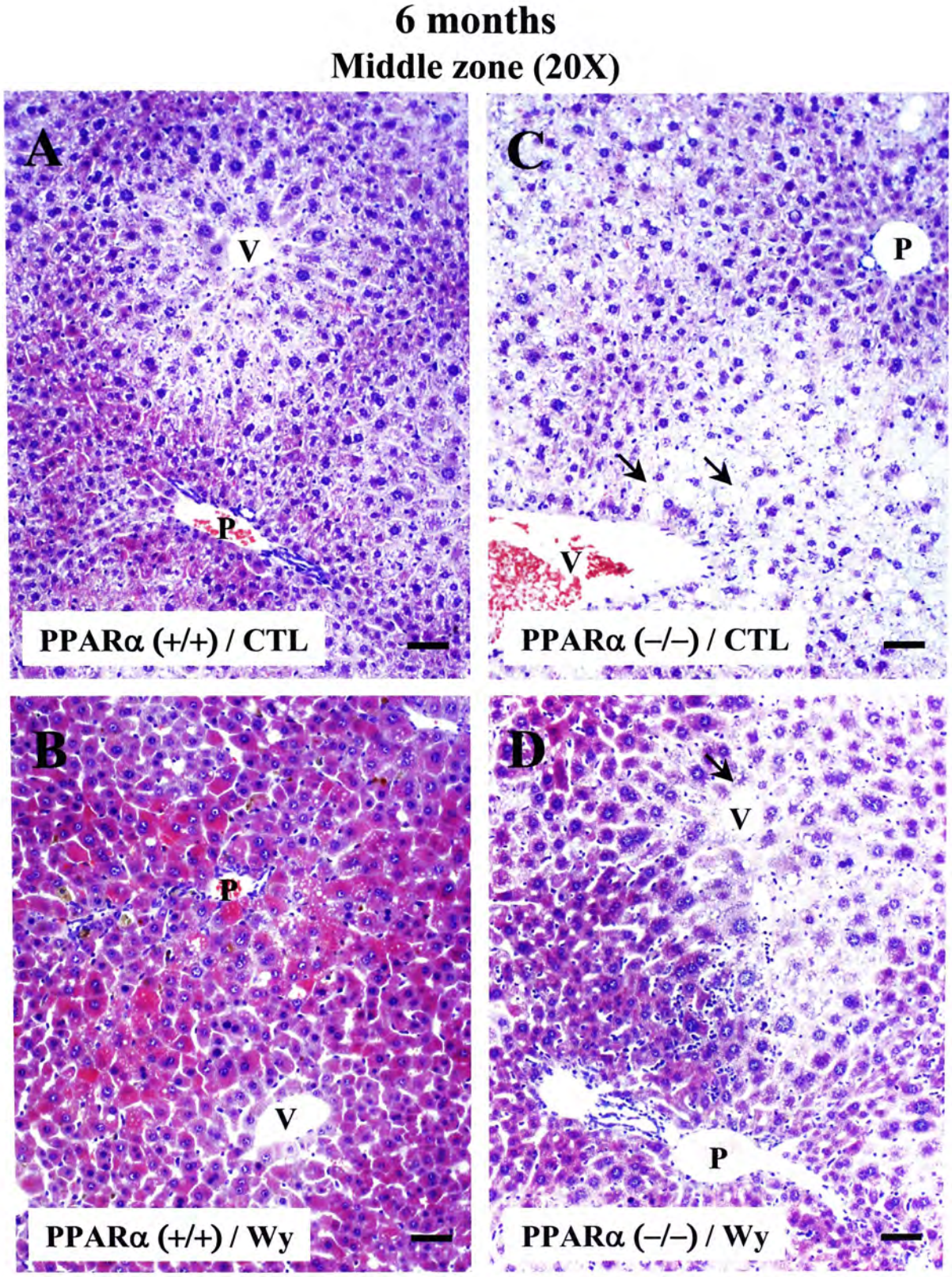

Figure 3.5.21. Hematoxylin and eosin staining of the middle zone (20X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 6 months treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Severe lipid accumulation (arrows) was found around the central vein region in both control and Wy-14,643 fed PPARα (-/-) mice. Irregular and trabecular cell clusters were observed in Wy-14,643 treated PPARα (+/+) mice. P, portal triad. V, central vein. Bars, 50 μm.

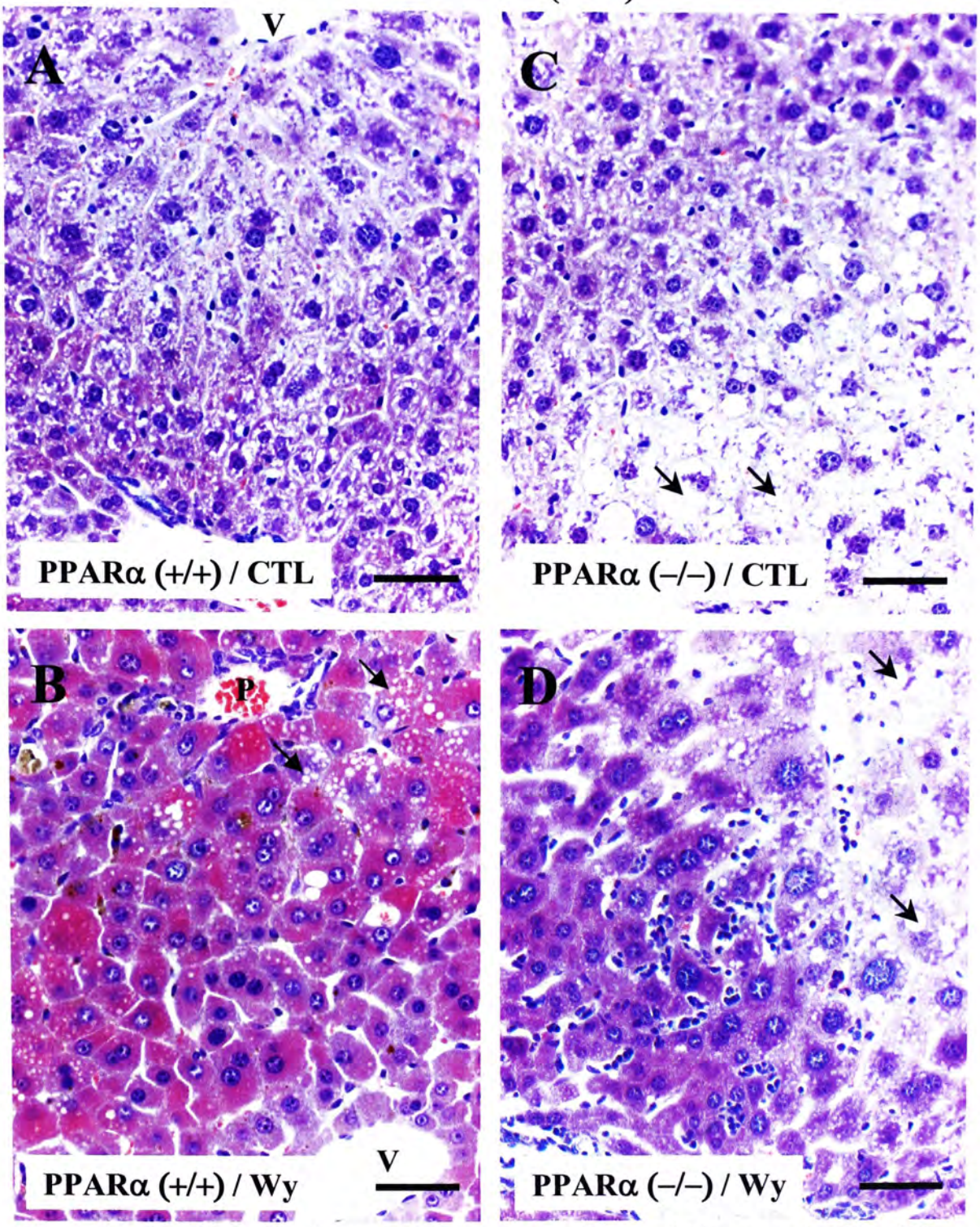

Figure 3.5.22. Hematoxylin and eosin staining of the middle zone (40X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 6 months treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Normal liver histology was found in PPARα (+/+) mice fed with control diet. Severe lipid accumulation (arrows) were found in both control and Wy-14,643 fed PPARα (-/-) mice. Lipid accumulation was also observed in PPARα (+/+) fed with control diet. P, portal triad. V, central vein. Bars, 50 μm.

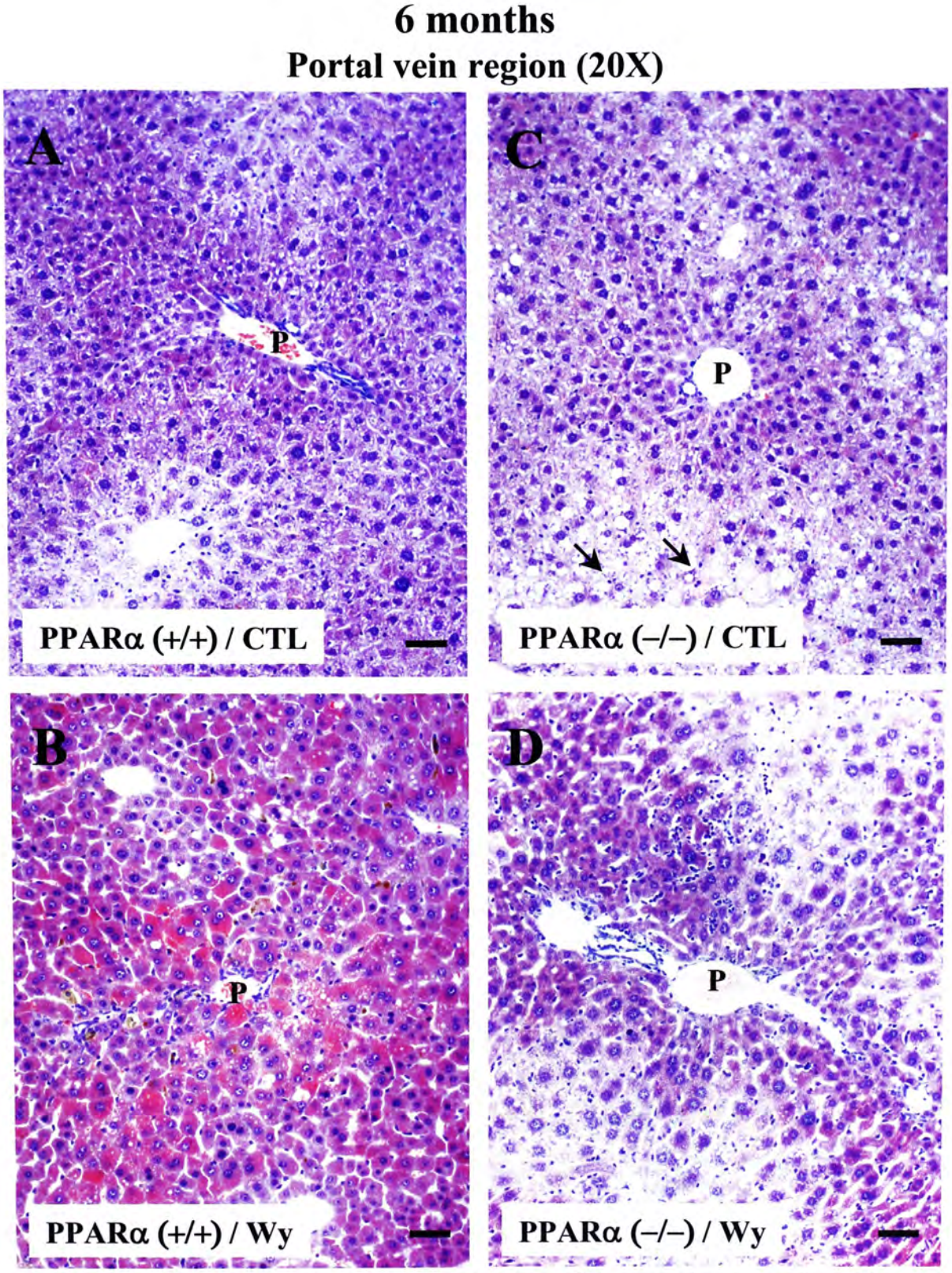

Figure 3.5.23. Hematoxylin and eosin staining of the portal vein region (20X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 6 months treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Normal liver histology was found in the portal vein region of both PPARα (+/+) and PPARα (-/-) mice fed with control diet. P, portal triad. Bars, 50 μm.

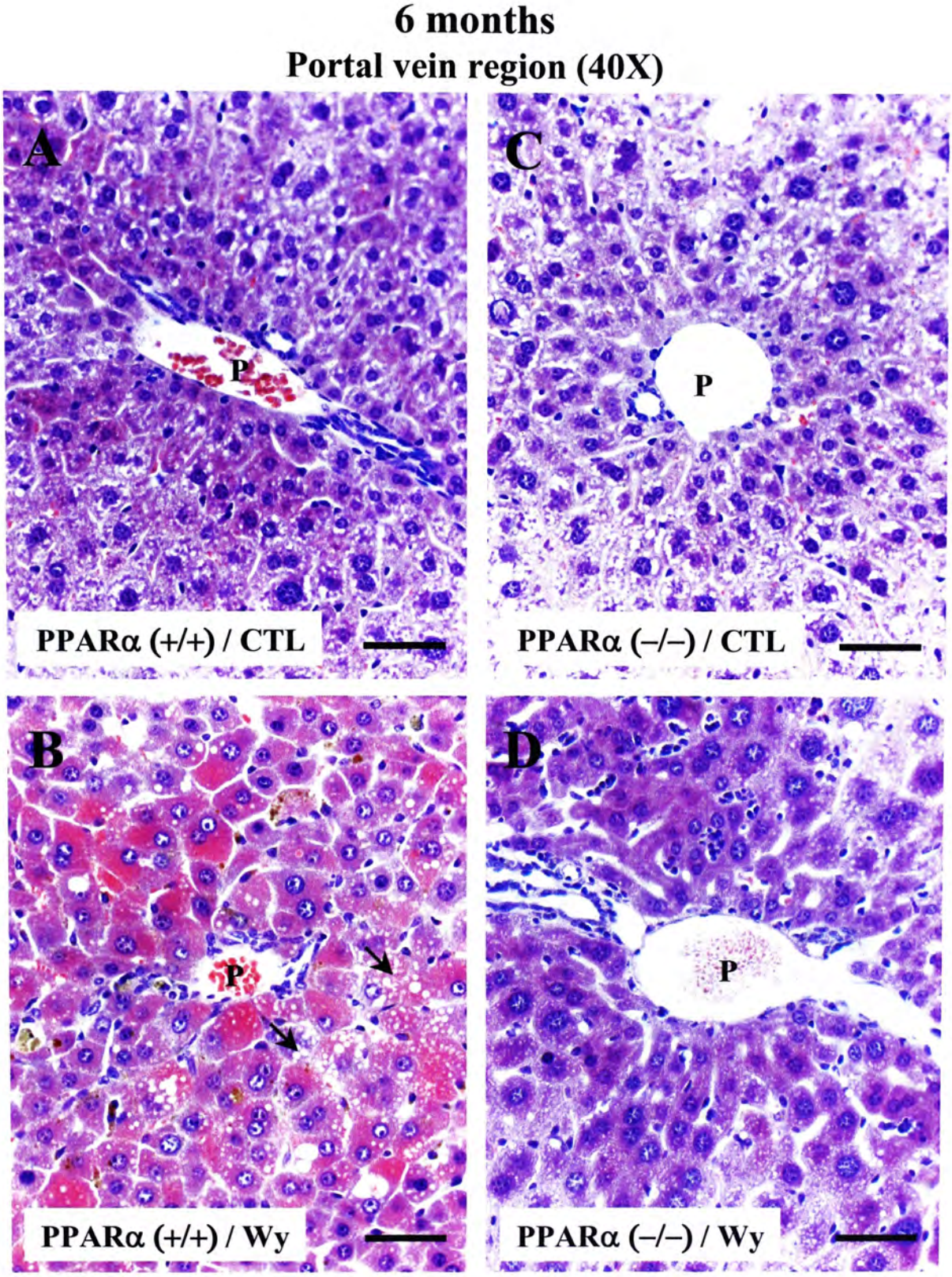

Figure 3.5.24. Hematoxylin and eosin staining of the portal vein region (40X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 6 months treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Normal liver histology was found in portal vein region both PPARα (+/+) and PPARα (-/-) mice fed with control diet. Lipid accumulation (arrows) was found in PPARα (+/+) mice fed with 0.1% Wy-14,643 diet. P, portal triad. Bars, 50 μm.

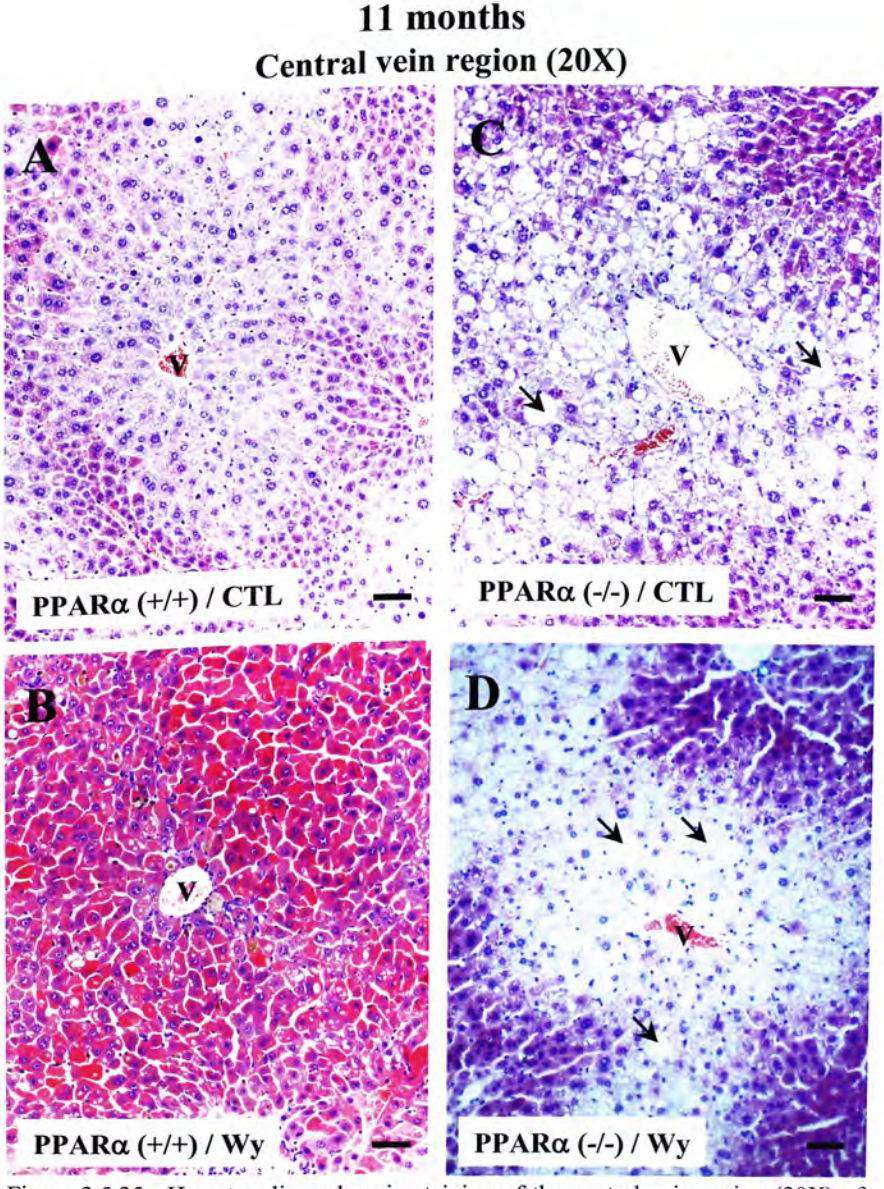

Figure 3.5.25. Hematoxylin and eosin staining of the central vein region (20X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 11 months treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Normal liver histology was found in PPARα (+/+) mice fed with control diet. Severe lipid accumulation (arrows) were found around the central vein region in both control and Wy-14,643 fed PPARα (-/-) mice. Irregular and trabecular cell clusters were observed in PPARα (+/+) fed with Wy-14643. V, central vein. Bars, 50 μm.

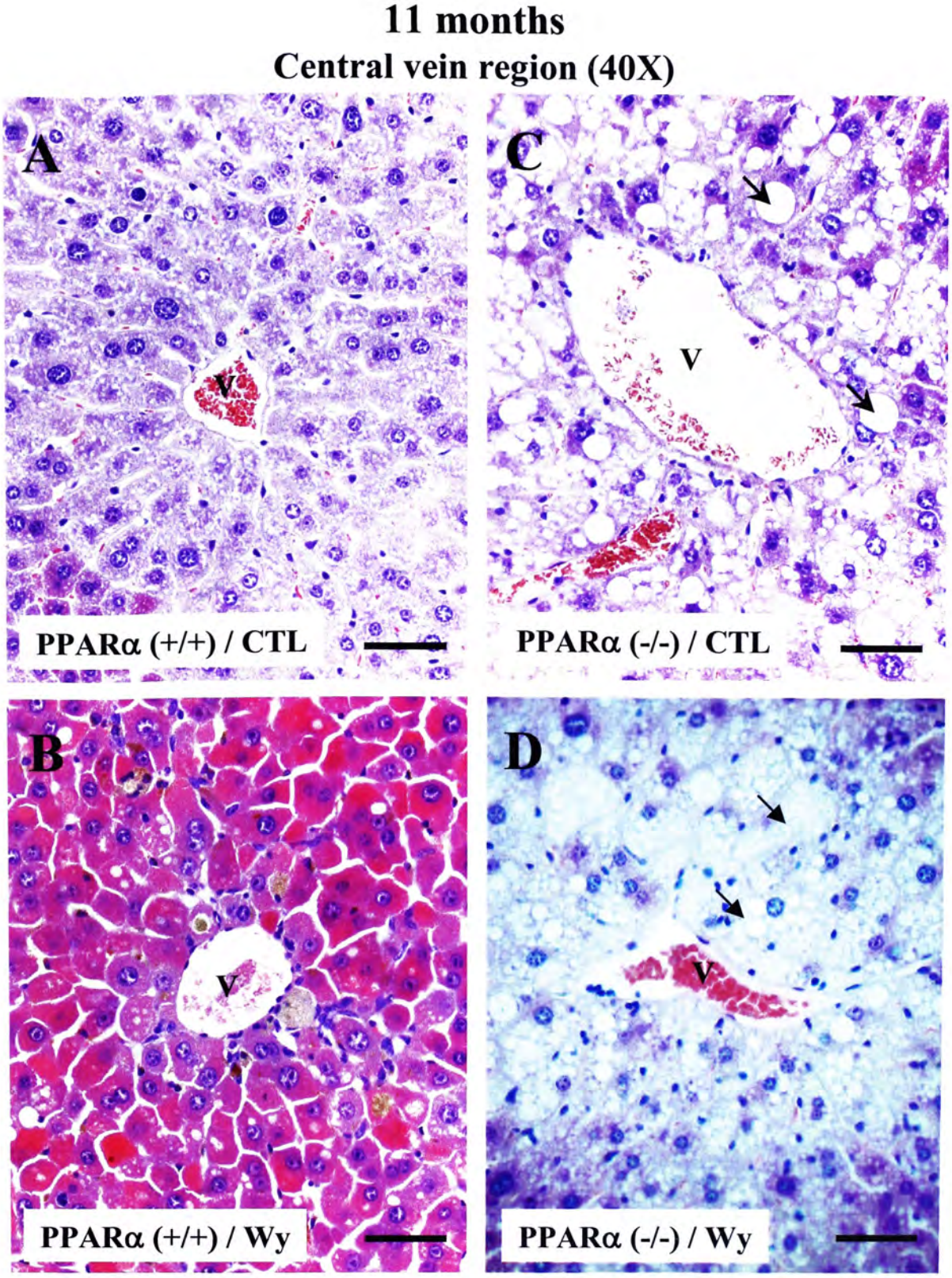

Figure 3.5.26. Hematoxylin and eosin staining of the central vein region (40X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 11 treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Normal liver histology was found in the central vein region of PPARα (+/+) mice fed with control diet. Severe lipid accumulation (arrows) was found around the central vein region in both control and Wy-14,643 fed PPARα (-/-) mice. Macrovesicular droplets were formed inside the hepatocytes and the nuclei were displaced to the periphery of the hypatocytes. V, central vein. Bars, 50 μm.

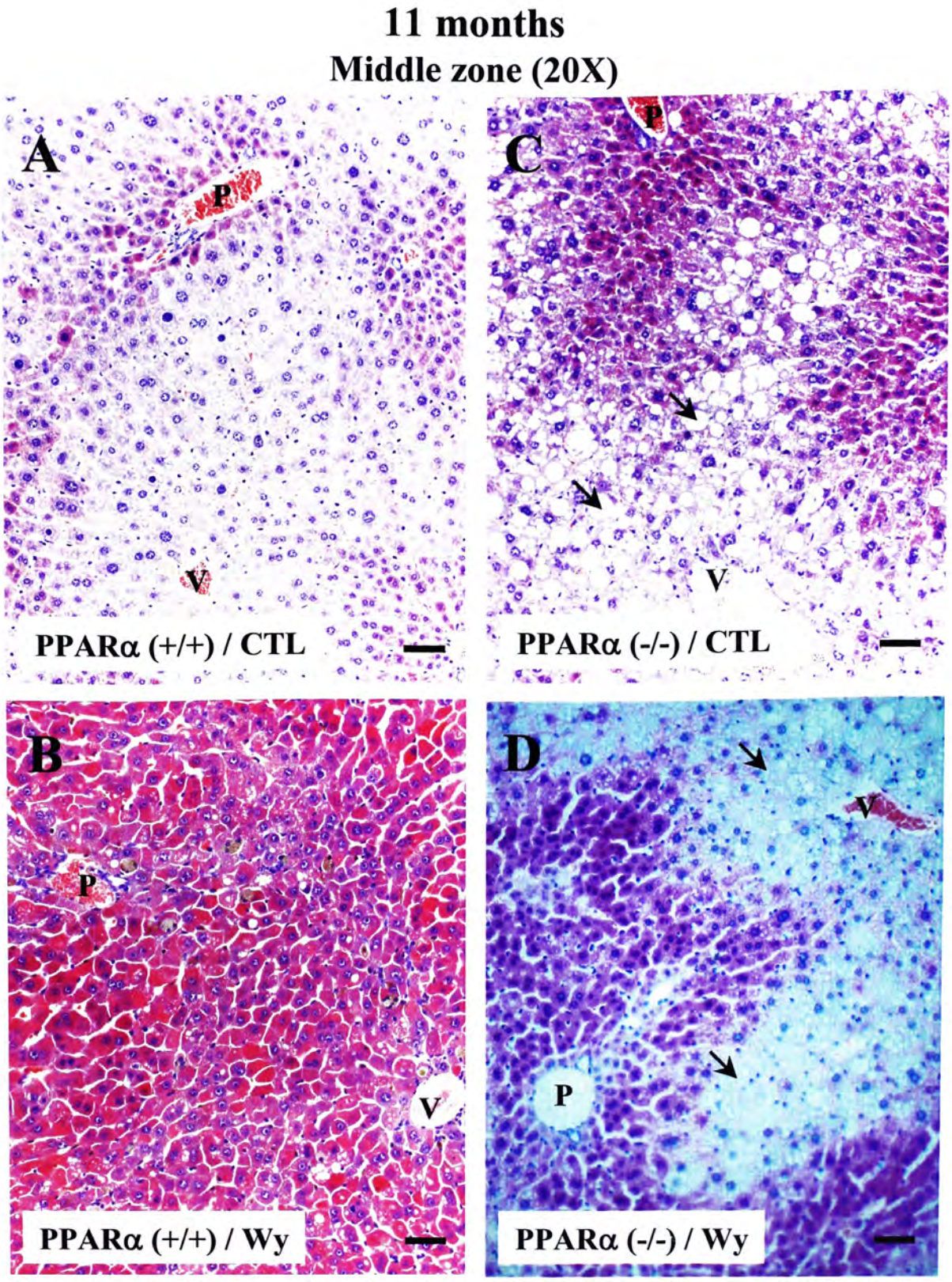

Figure 3.5.27. Hematoxylin and eosin staining of the middle zone (20X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 11 months treatment with a 0.0% Wy-14,643 (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Normal liver histology was found in the central vein region of PPARα (+/+) fed with control diet while irregular and trabecular cell clustes were observed in Wy-14,643 fed PPARα (+/+) mice. Lipid accumulation (arrows) was found around the central vein region in both control and Wy-14,643 fed PPARα (-/-) mice. P, portal triad. V, central vein. Bars, 50 μm.

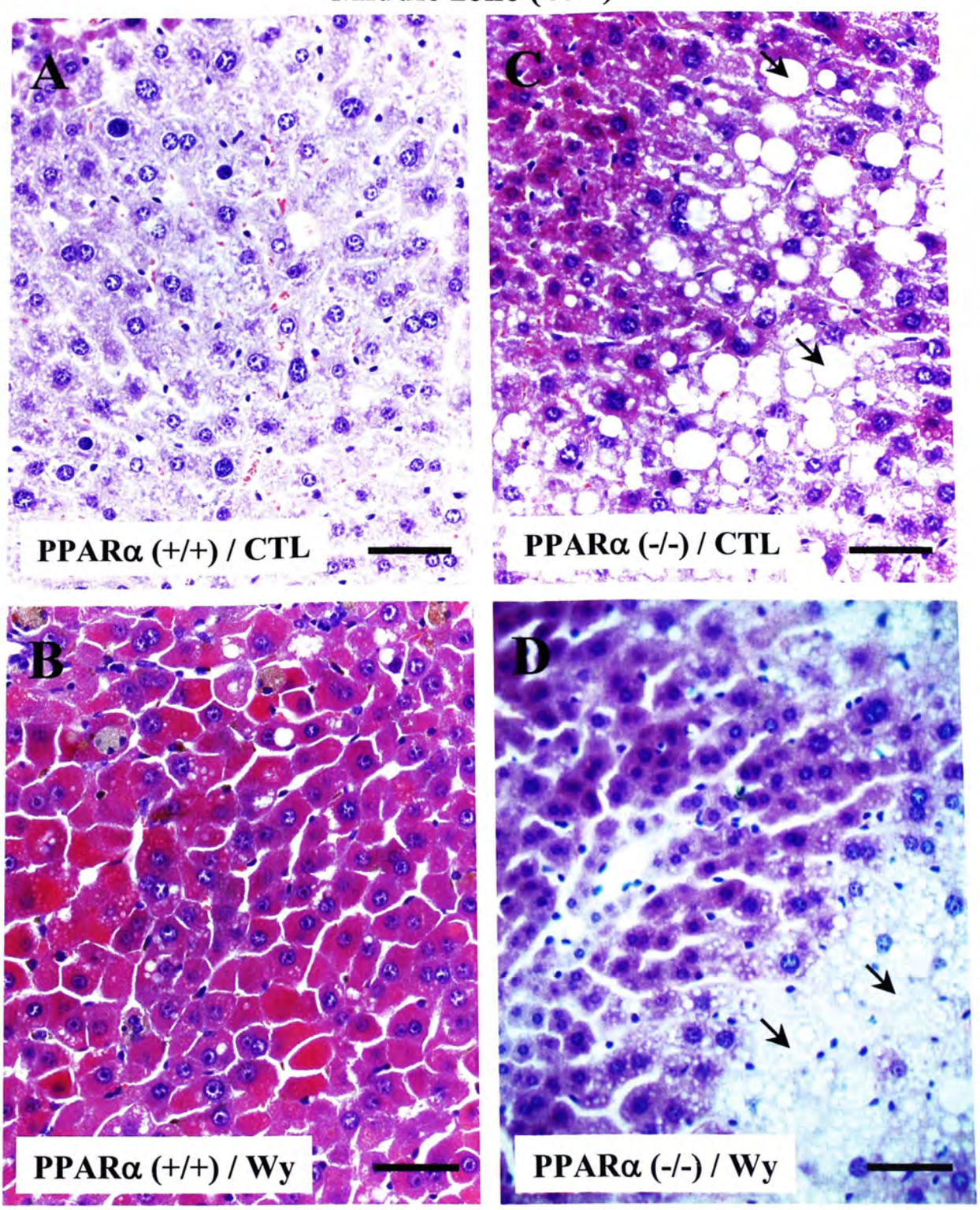

Figure 3.5.28. Hematoxylin and eosin staining of the middle zone (40X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 11 months treatment with a 0.0% Wy-14,643 (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Severe lipid accumulation (arrows) were found around in both control and Wy-14,643 fed PPARα (-/-) mice. Macrovesicular droplets were formed inside the hepatocytes and the nuclei were displaced to the periphery of the hepatocytes. Bars, 50 μm.

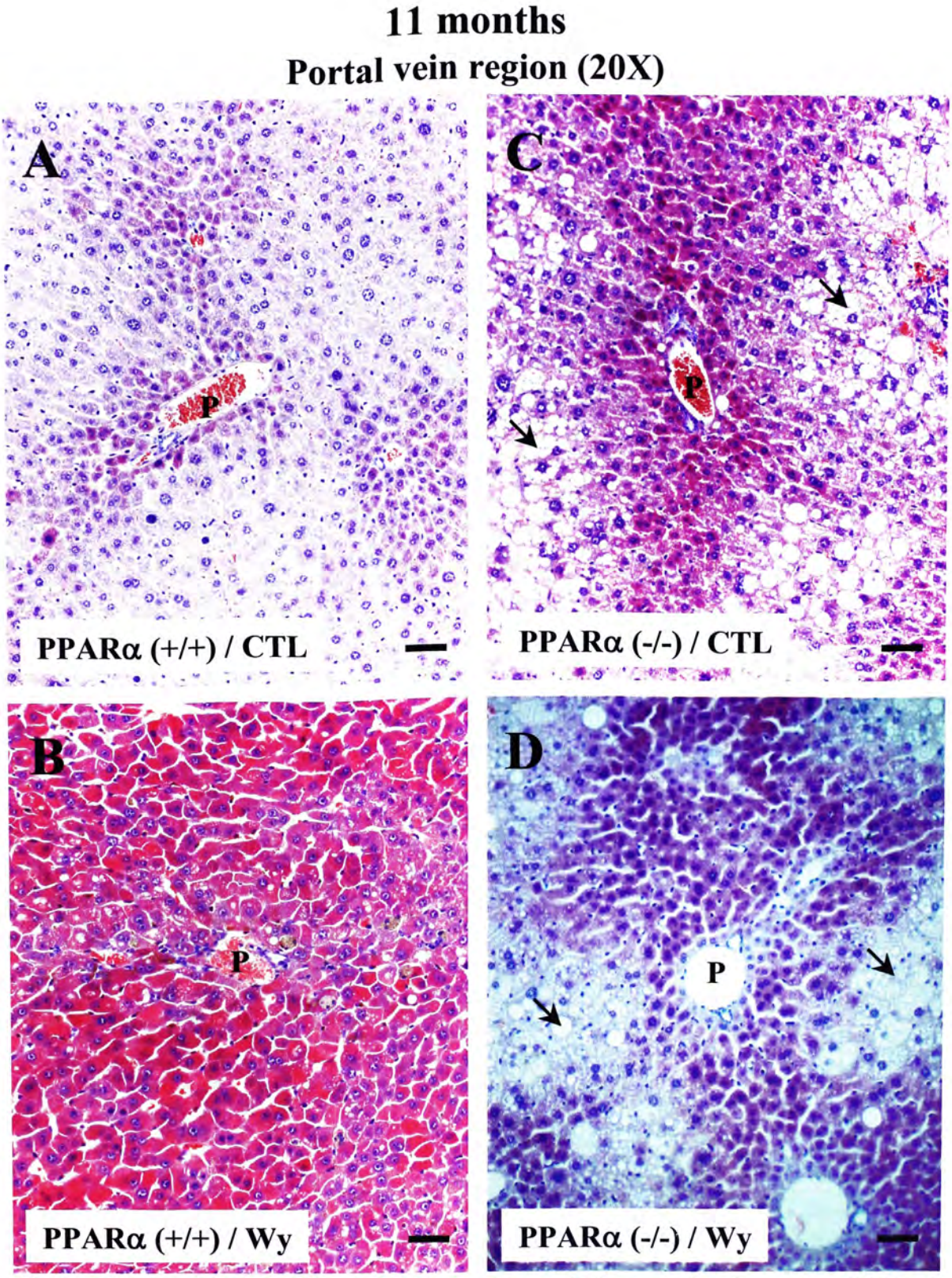

Figure 3.5.29. Hematoxylin and eosin staining of the portal vein region (20X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 11 months treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Severe lipid accumulation (arrows) was found around the central vein region in both control and Wy-14,643 fed PPARα (-/-) mice. P, portal triad. Bars, 50 μm.

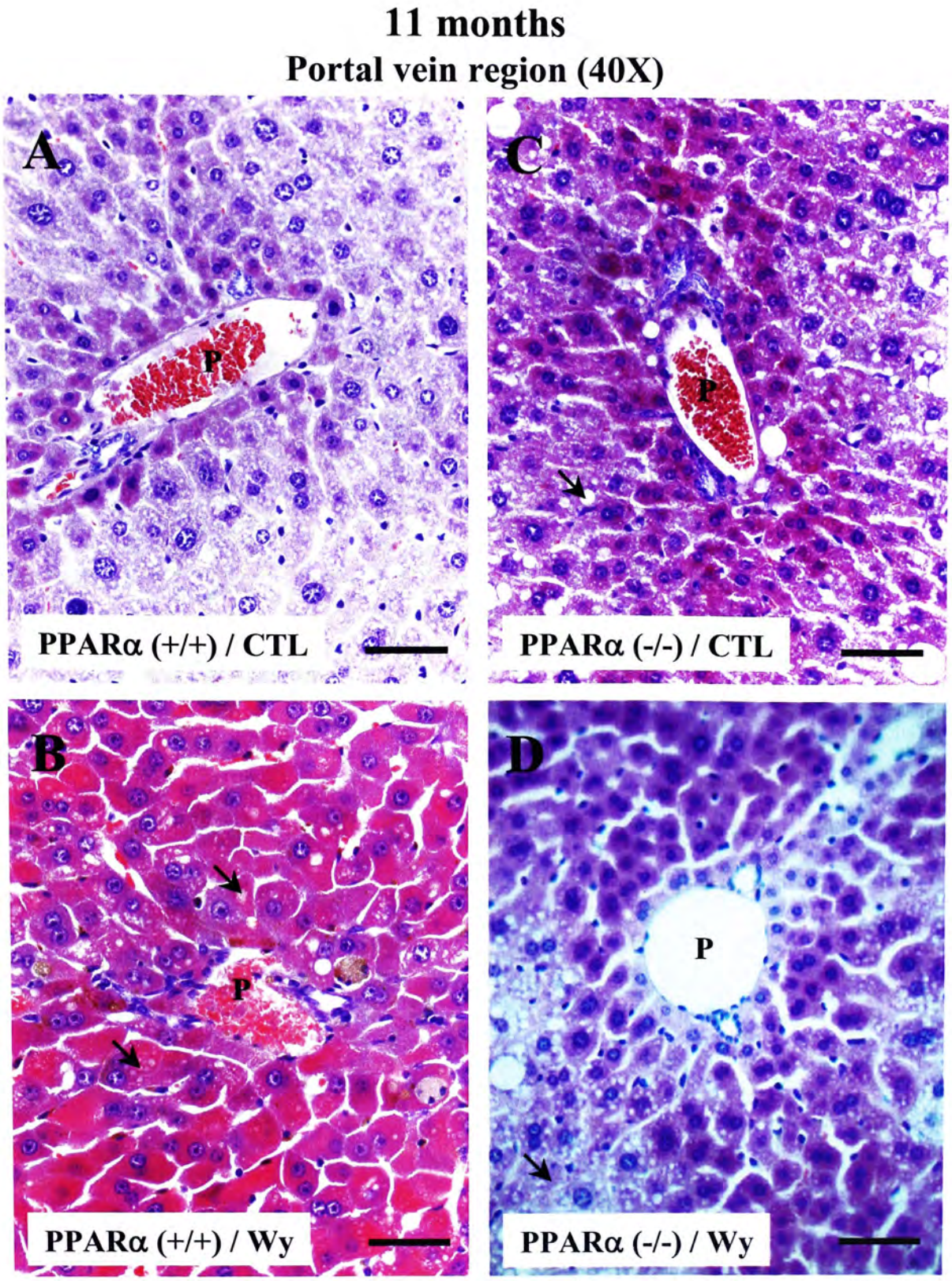

Figure 3.5.30. Hematoxylin and eosin staining of the portal vein region (40X) of liver sections (5 μm) taken from PPARα (+/+) and PPARα (-/-) mice after 11 months treatment with a 0.0% Wy-14,643 (control diet) (panels A, B) or a 0.1% Wy-14,643 diet (panels C, D) (n=3). Lipid accumulation (arrows) was found in both PPARα (+/+) and PPARα (-/-) mice fed with 0.1% Wy-14,643 diet and PPARα (-/-) mice fed with control diet. P, portal triad. Bars, 50 μm.

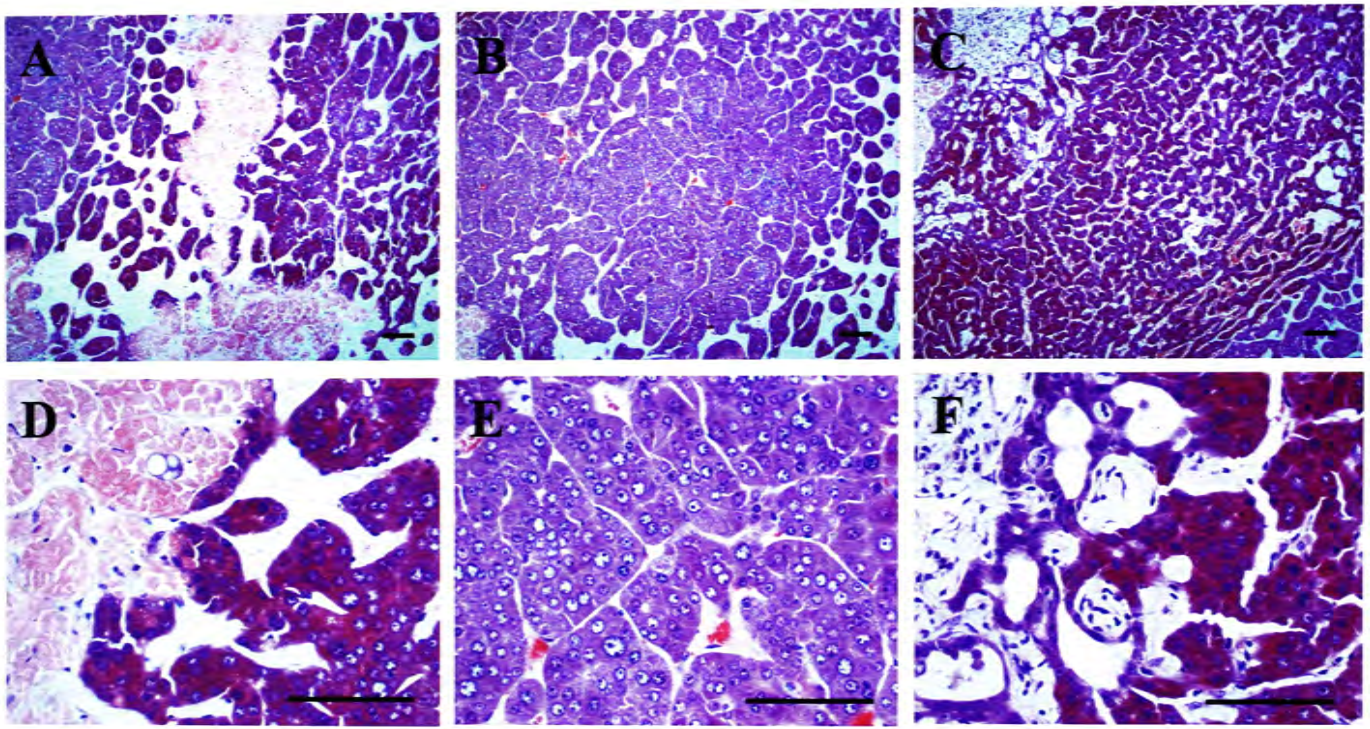

Figure 3.5.31. Hematoxylin and eosin staining of liver sections (5 μm) taken from PPARα (+/+) after 11 months treatment with a 0.1% Wy-14,643 diet (10X: panels A, B, C; 40X: panels D, E, F). Hepatocytes without nuclei (panels A, D) and trabecular cell clusters were observed (panels B, E). Dark nucleus and invasion of blood in sinusoidal spaces were also shown (panels C, F). Bars, 100 μm.

3.6 Reverse transcription (RT) of mRNA and non-fluorescent PCR (non-fluoroDD PCR)

Since there are twelve different anchored primers (AP) and twenty different arbitrary primers (ARP), a total of 240 different combinations of AP and ARP can be used for the RT-PCR. However, not all the combinations will generate our desired differential display patterns (fluoroDD patterns) (Figure 2.10.1). In order to screen out the primer pairs which will show our desired fluoroDD patterns, reverse transcription (RT) of mRNA with non-fluorescent PCR (non-fluoroDD PCR), a faster and more economic way, were performed in this study.

Forty-three different primer combinations (AP1 & ARP2, ARP3, ARP6, ARP10, ARP16 and ARP20; AP2+ARP1, ARP3, ARP10, ARP12, ARP16, ARP18 and ARP19; AP3 & ARP3; AP5 & ARP1, ARP2, ARP3, ARP10, ARP12 and ARP18; AP6 & ARP1, ARP4, ARP5, ARP8, ARP12 and ARP14; AP7 & ARP10 and ARP15; AP10 & ARP1, ARP6, ARP10, ARP12, ARP13 and ARP16; AP11 & ARP1, ARP3, ARP5, ARP8, ARP12, ARP17, ARP19 and ARP20; and AP12 & ARP2) were used to perform non-fluoroDD RT-PCR. Twenty-six primer pairs including AP1 & ARP2, ARP3 and ARP20 (Figure 3.6.1); AP2 & ARP1, ARP10, ARP12, ARP18 and ARP19 (Figure 3.6.2); AP3 & ARP3 (Figure 3.6.3); AP5 & ARP1, ARP2, ARP3, ARP10 and ARP12 (Figure 3.6.4); AP6 & ARP1, ARP4 and ARP14 (Figure 3.6.5); AP7 & ARP15 (Figure 3.6.6); AP10 & ARP1, ARP12 and ARP13 (Figure 3.6.7); AP11 & ARP1, ARP3, ARP12 and ARP19 (Figure 3.6.8); and AP12 & ARP2 (Figure 3.6.9) showed differential display patterns, while the others did not show obvious differential display patterns or gave inconsistent patterns (Table 3.6.1). Eleven primer pairs, including AP1 & ARP2 (Figure 3.6.1); AP2 & ARP18 and ARP19 (Figure 3.6.2);

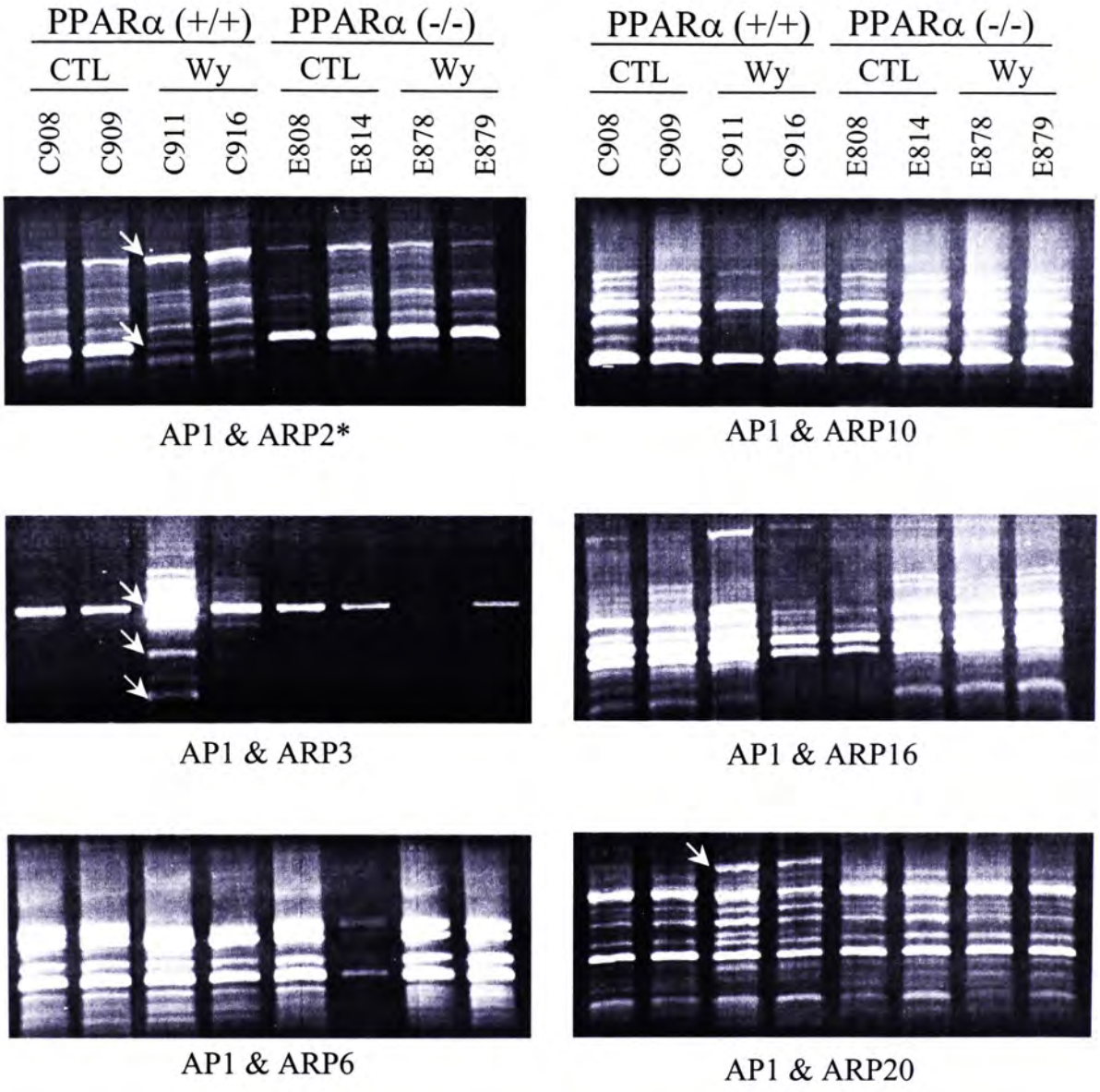

Figure 3.6.1. Preliminary screening for differential display patterns using non-fluorescent AP1 in combinations with ARP2, ARP3, ARP6, ARP10, ARP16 and ARP20. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP1 primer and amplified by PCR using 3'-AP1 and 5'-ARPs (ARP2, ARP3, ARP6, ARP10, ARP16 and ARP20). Each treatment group consists of two mice and the PCR products were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. Arrow heads indicate the Wy-14,643 responsive and PPARα-dependent cDNA fragments. *Primer pair used for subsequent fluorescent differential display analysis.

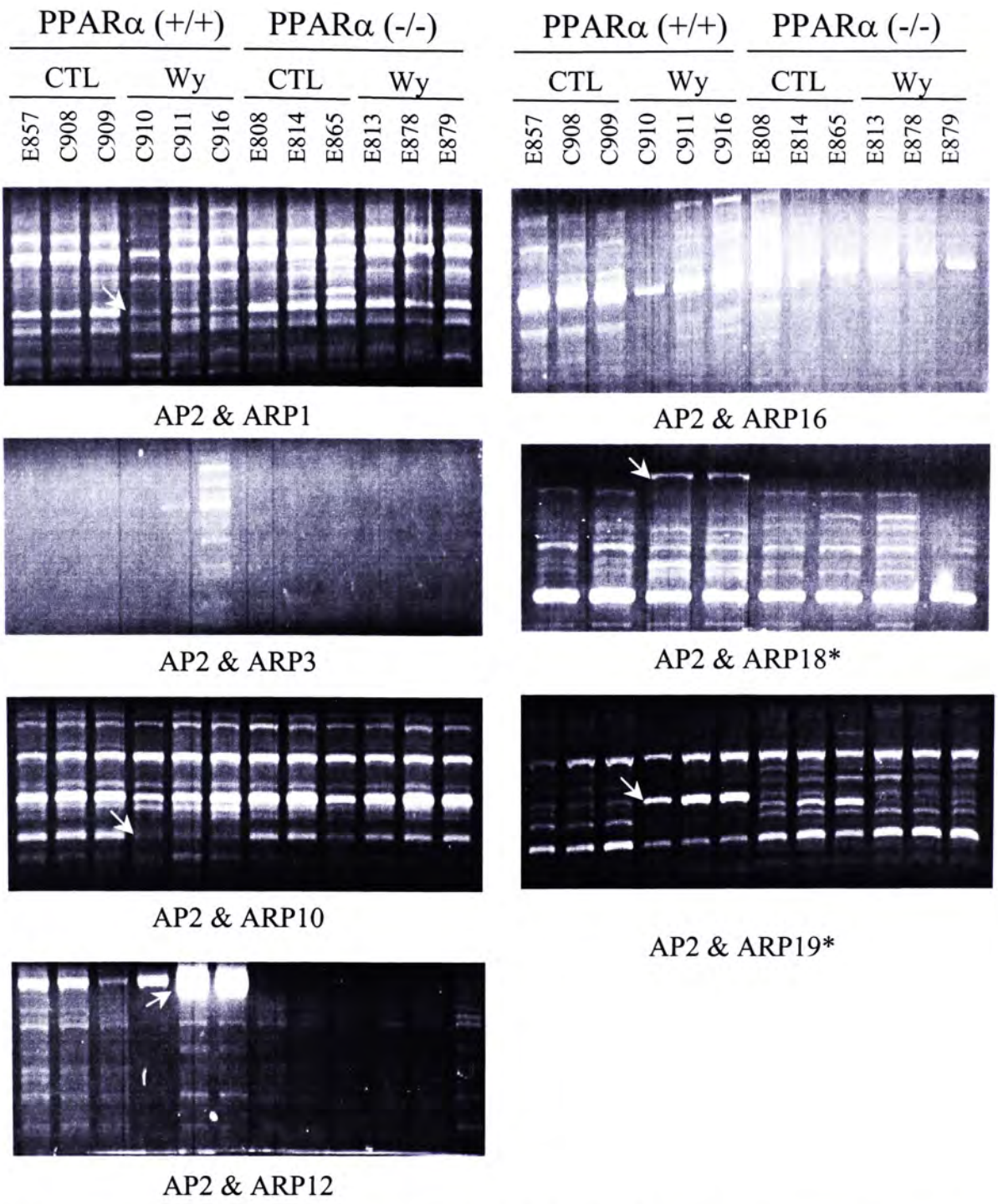

Figure 3.6.2. Preliminary screening for differential display patterns using non-fluorescent AP2 in combinations with ARP1, ARP3, ARP10, ARP12, ARP16, ARP18 and ARP19. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP2 primer and amplified by PCR using 3'-AP2 and 5'-ARPs (ARP1, ARP3, ARP10, ARP12, ARP16, ARP18 and ARP19). Each treatment group consists of two or three mice and the PCR products were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. Arrow heads indicate the Wy-14,643 responsive and PPARα-dependent cDNA fragments. *Primer pairs used for subsequent fluorescent differential display analysis.

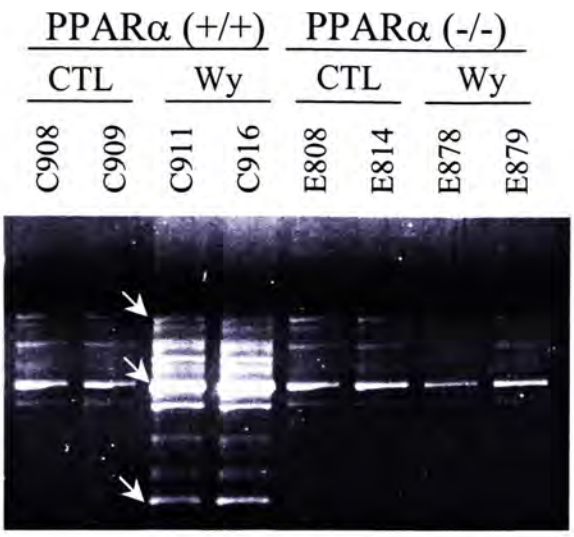

Figure 3.6.3. Preliminary screening for differential display patterns using non-fluorescent AP3 in combination with ARP3. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP3 primer and amplified by PCR using 3'-AP3 and 5'-ARP3. Each treatment group consists of two mice and the PCR products were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. Arrow heads indicate the Wy-14,643 responsive and PPARα-dependent cDNA fragments. *Primer pair used for subsequent fluorescent differential display analysis.

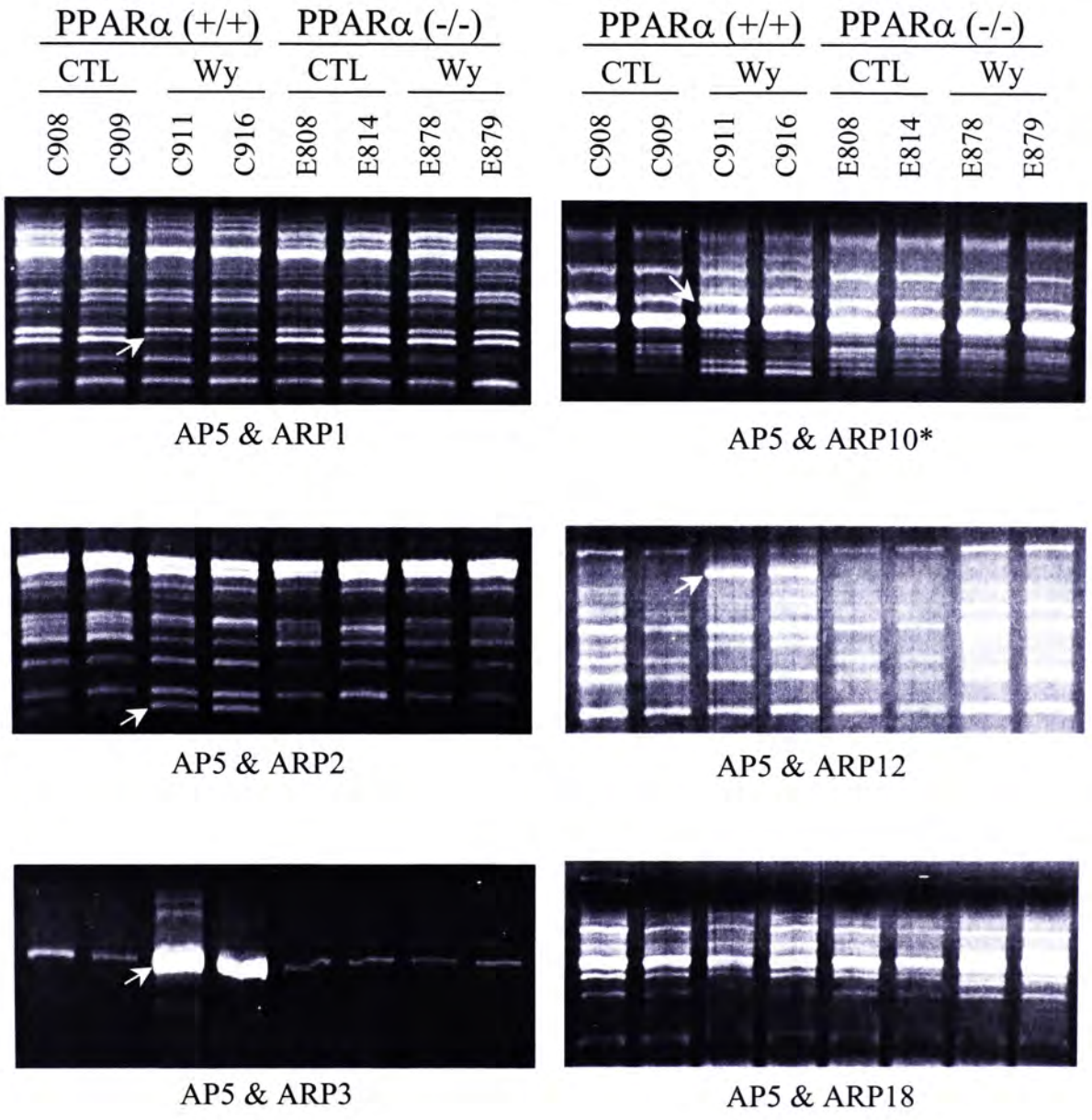

Figure 3.6.4. Preliminary screening for differential display patterns using non-fluorescent AP5 in combinations with ARP1, ARP2, ARP3, ARP10, ARP12 and ARP18. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP5 primer and amplified by PCR using 3'-AP5 and 5'-ARPs (ARP1, ARP2, ARP3, ARP10, ARP12 and ARP18). Each treatment group consists of two mice and the PCR products were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. Arrow heads indicate the Wy-14,643 responsive and PPARα-dependent cDNA fragments. *Primer pair used for subsequent fluorescent differential display analysis.

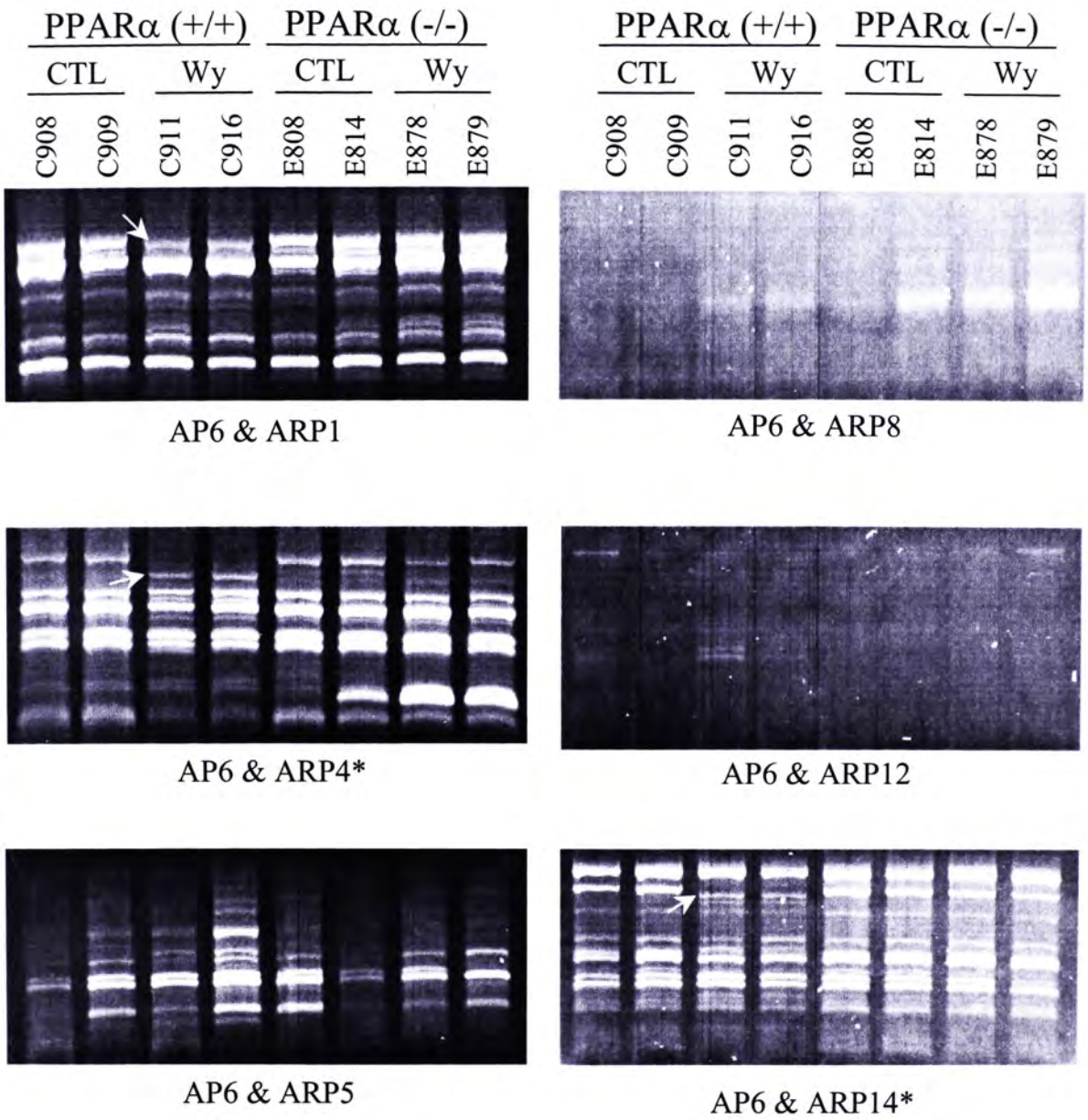

Figure 3.6.5. Preliminary screening for differential display patterns using non-fluorescent AP6 in combinations with ARP1, ARP4, ARP5, ARP8, ARP12 and ARP14. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP6 primer and amplified by PCR using 3'-AP6 and 5'-ARPs (ARP1, ARP4, ARP5, ARP8, ARP12 and ARP14). Each treatment group consists of two mice and the PCR products were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. Arrow heads indicate the Wy-14,643 responsive and PPARα-dependent cDNA fragments. *Primer pairs used for subsequent fluorescent differential display analysis.

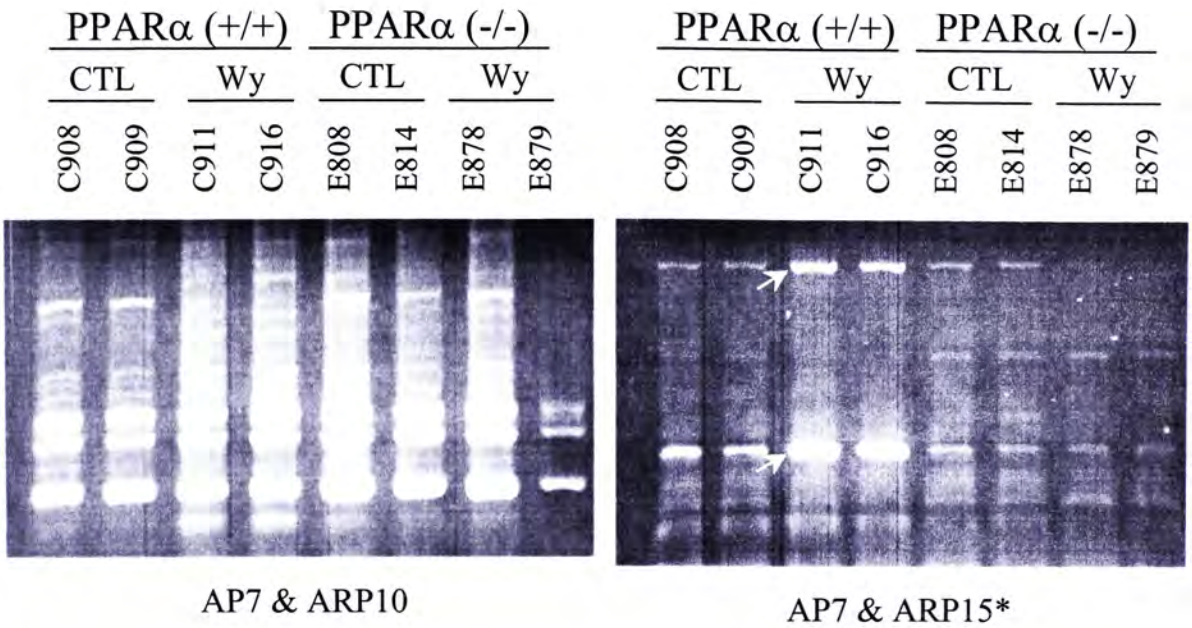

Figure 3.6.6. Preliminary screening for differential display patterns using non-fluorescent AP7 in combinations with ARP10 and ARP15. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP7 primer and amplified by PCR using 3'-AP7 and 5'-ARPs (ARP10 and ARP15). Each treatment group consists of two mice and the PCR products were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. Arrow heads indicate the Wy-14,643 responsive and PPARα-dependent cDNA fragments. *Primer pair used for subsequent fluorescent differential display analysis.

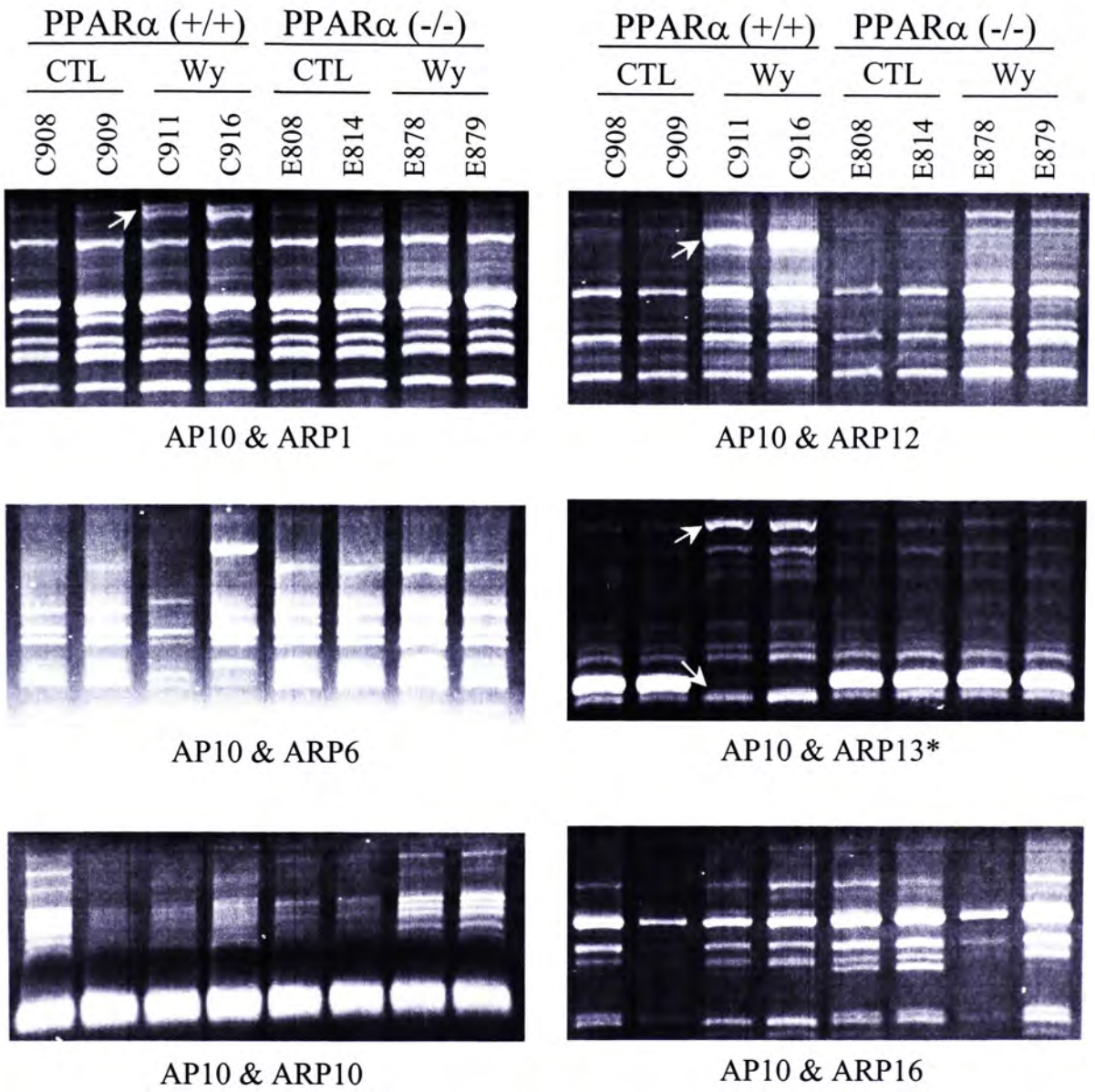

Figure 3.6.7. Preliminary screening for differential display patterns using non-fluorescent AP10 in combinations with ARP1, ARP6, ARP10, ARP12, ARP13 and ARP16. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP10 primer and amplified by PCR using 3'-AP10 and 5'-ARPs (ARP1, ARP6, ARP10, ARP12, ARP13 and ARP16). Each treatment group consists of two mice and the PCR products were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. Arrow heads indicate the Wy-14,643 responsive and PPARα-dependent cDNA fragments. *Primer pair used for subsequent fluorescent differential display analysis.

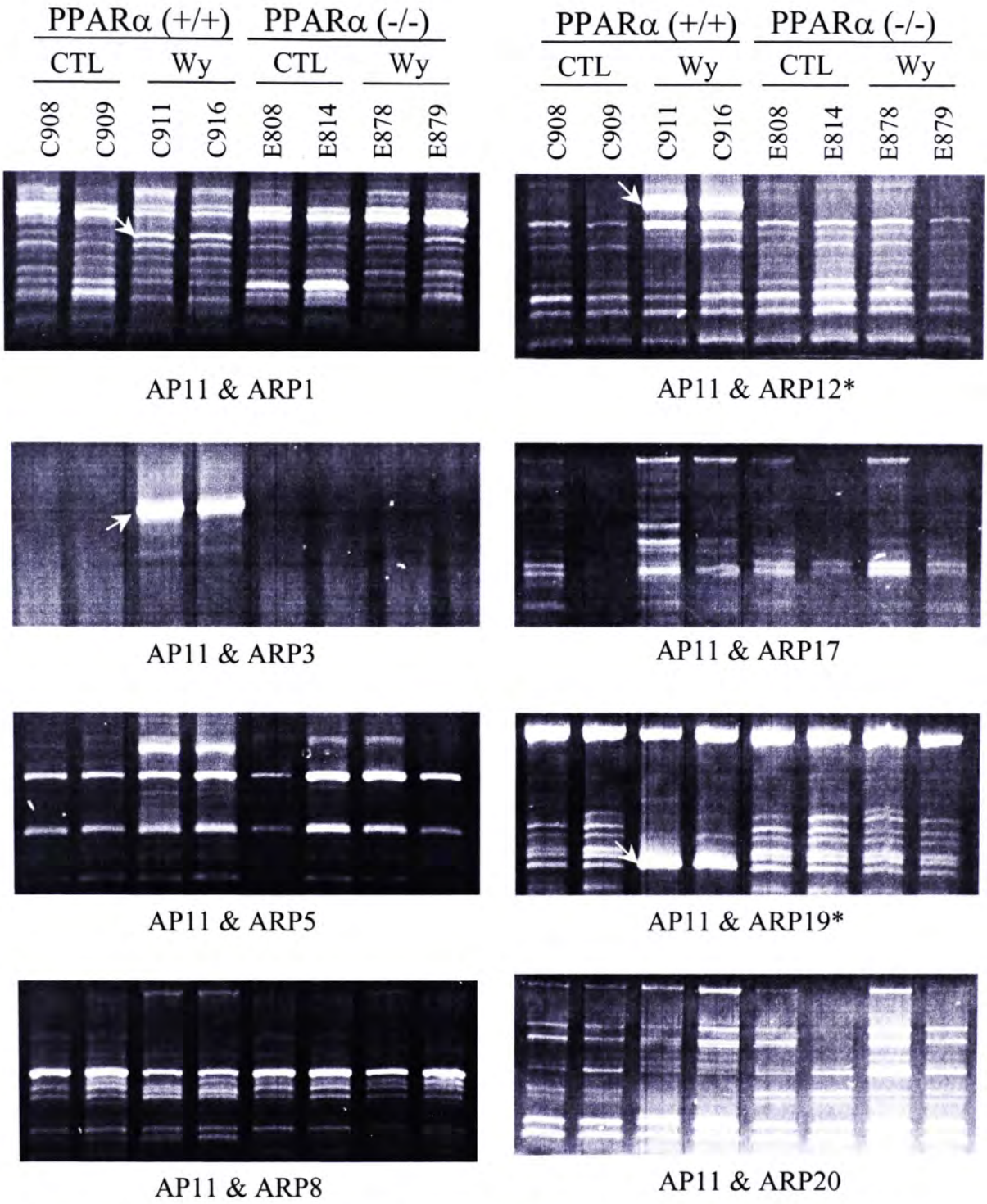

Figure 3.6.8. Preliminary screening for differential display patterns using non-fluorescent AP11 in combinations with ARP1, ARP3, ARP5, ARP8, ARP12, ARP17, ARP19 and ARP20. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP11 primer and amplified by PCR using 3'-AP11 and 5'-ARPs (ARP1, ARP3, ARP5, ARP8, ARP12, ARP17, ARP19 and ARP20). Each treatment group consists of two mice and the PCR products were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. Arrow heads indicate the Wy-14,643 responsive and PPARα-dependent cDNA fragments. *Primer pairs used for subsequent fluorescent differential display analysis.

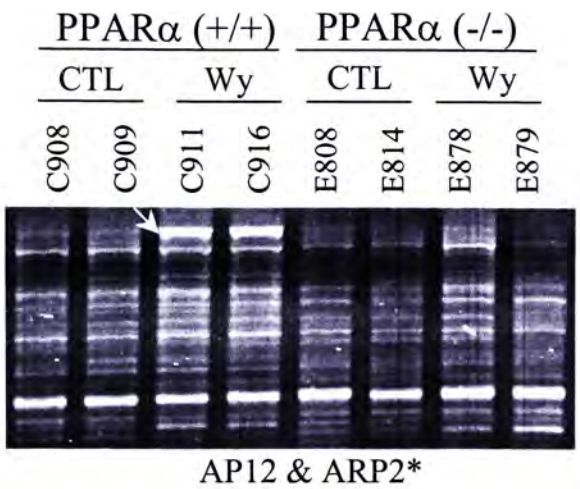

Figure 3.6.9. Preliminary screening for differential display patterns using non-fluorescent AP12 in combinations with ARP2. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP12 primer and amplified by PCR using 3'-AP12 and 5'-ARP2. Each treatment group consists of two mice and the PCR products were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. Arrow head indicates the Wy-14,643 responsive and PPARα-dependent cDNA fragments. *Primer pair used for subsequent fluorescent differential display analysis.

Table 3.6.1. Combinations of anchored primers (APs) and arbitrary primers (ARPs) used for non-fluoroDD RT-PCR

	ARP1	ARP2	ARP3	ARP4	ARP5	ARP6	ARP7	ARP8	ARP9	ARP10	ARP11	ARP12	ARP13	ARP14	ARP15	ARP16	ARP17	ARP18	ARP19	ARP20
AP1		1-2*†	1-3*			1-6				1-10						1-16				1-20*
AP2	2-1*		2-3							2-10*		2-12*				2-16		2-18*†	2-19*†	
AP3			3-3*†																	
AP4																				
AP5	5-1*	5-2*	5-3*							5-10*†		5-12*						5-18		
AP6	6-1*			6-4*†	6-5			6-8				6-12		6-14*†						
AP7										7-10					7-15*†					
AP8																				
AP9																				
AP10	10-1*					10-6				10-10		10-12*	10-13*†			10-16				
AP11	11-1*		11-3*		11-5			11-8				11-12*†					11-17		11-19*†	11-20
AP12		12-2*†																		

Forty-three AP-ARP pairs which are highlighted in gray were chosen randomly for preliminary non-fluoroDD RT-PCR. *The AP-ARP pairs displayed differential expression patterns and †the AP-ARP pairs selected for subsequent fluoroDD RT-PCR.

AP3 & ARP3 (Figure 3.6.3); AP5 & ARP10 (Figure 3.6.4); AP6 & ARP4 and ARP14 (Figure 3.6.5); AP7 & ARP15 (Figure 3.6.6); AP10 & ARP13 (Figure 3.6.7); AP11 & ARP19 (Figure 3.6.8); and AP12 & ARP2 (Figure 3.6.9), which showed differential display patterns in the fast screening of non-fluoro PCR were used for fluorescent RT-PCR (fluoroDD RT-PCR).

3.7 Reverse transcription (RT) of mRNA and fluorescent PCR (fluoroDD PCR)

Eleven fluoroDD gels were performed with eleven AP-ARP primer pairs which yield desired differential display patterns. They included gels AA (AP1 & ARP2) (Figure 3.7.1), AB (AP3 & ARP3) (Figure 3.7.2), AC (AP2 & ARP19) (Figure 3.7.3), AD (AP2 & ARP18) (Figure 3.7.4), AF (AP10 & ARP13) (Figure 3.7.5), AH (AP11 & ARP19) (Figure 3.7.6), AI (AP6 & ARP4) (Figure 3.7.7), AJ (AP6 & ARP14) (Figure 3.7.8), AL (AP7 & ARP15) (Figure 3.7.9), AO (AP5 & ARP10) (Figure 3.7.10) and AP (AP12 & ARP2) (Figure 3.7.11).

One hundred fifty-seven fluoroDD fragments which showed our desired differential display patterns were excised from the eleven fluoroDD gels (Tables 3.7.1-3.7.10). All together, forty-one fluoroDD fragments were down-regulated by 0.1% Wy-14,643 in PPARα (+/+) group, whereas the other one hundred and sixteen fluoroDD fragments were up-regulated by 0.1% Wy-14,643 in PPARα (+/+) mice. For gel AA, sixteen bands were excised (AA1, AA2, AA3, AA4, AA5, AA6, AA7, AA8, AA9, AA10, AA11, AA12, AA14, AA15, AA16 and AA17) (Figure 3.7.1) (Table 3.7.1). For gel AB, thirty-four bands were excised (AB1, AB2, AB3, AB4, AB5, AB6, AB7, AB8, AB9, AB10, AB11, AB12, AB13, AB14, AB15, AB16,

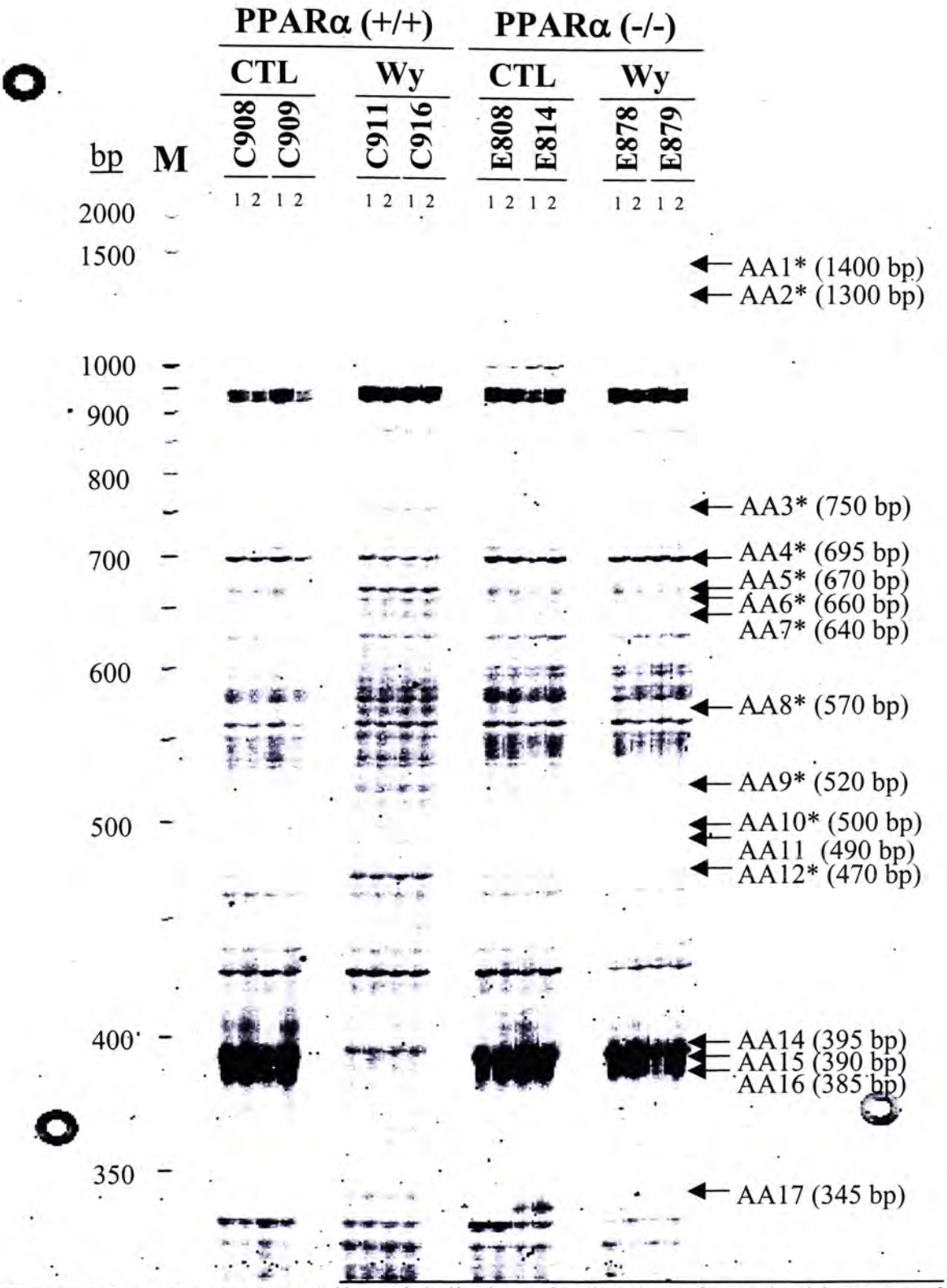

Figure 3.7.1. Fluorescent differential display of gel AA performed with AP1 and ARP2. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP1 and duplicate PCR reactions were performed for each mouse with 3'-TMR-labeled AP1 (ACGACTCACTATAGGGCTTTTTTTTTTTGA) and 5'-ARP2 (ACAATTTCACACAGGAGCTAGCATGG). The TMR-labeled fluorescent PCR products were run on a 5.6% denaturing polyacrylamide gel. In gel AA, sixteen cDNA fragments showing differential display patterns were excised. *Fragments selected for reamplification. M, TMR-labeled molecular weight DNA marker.

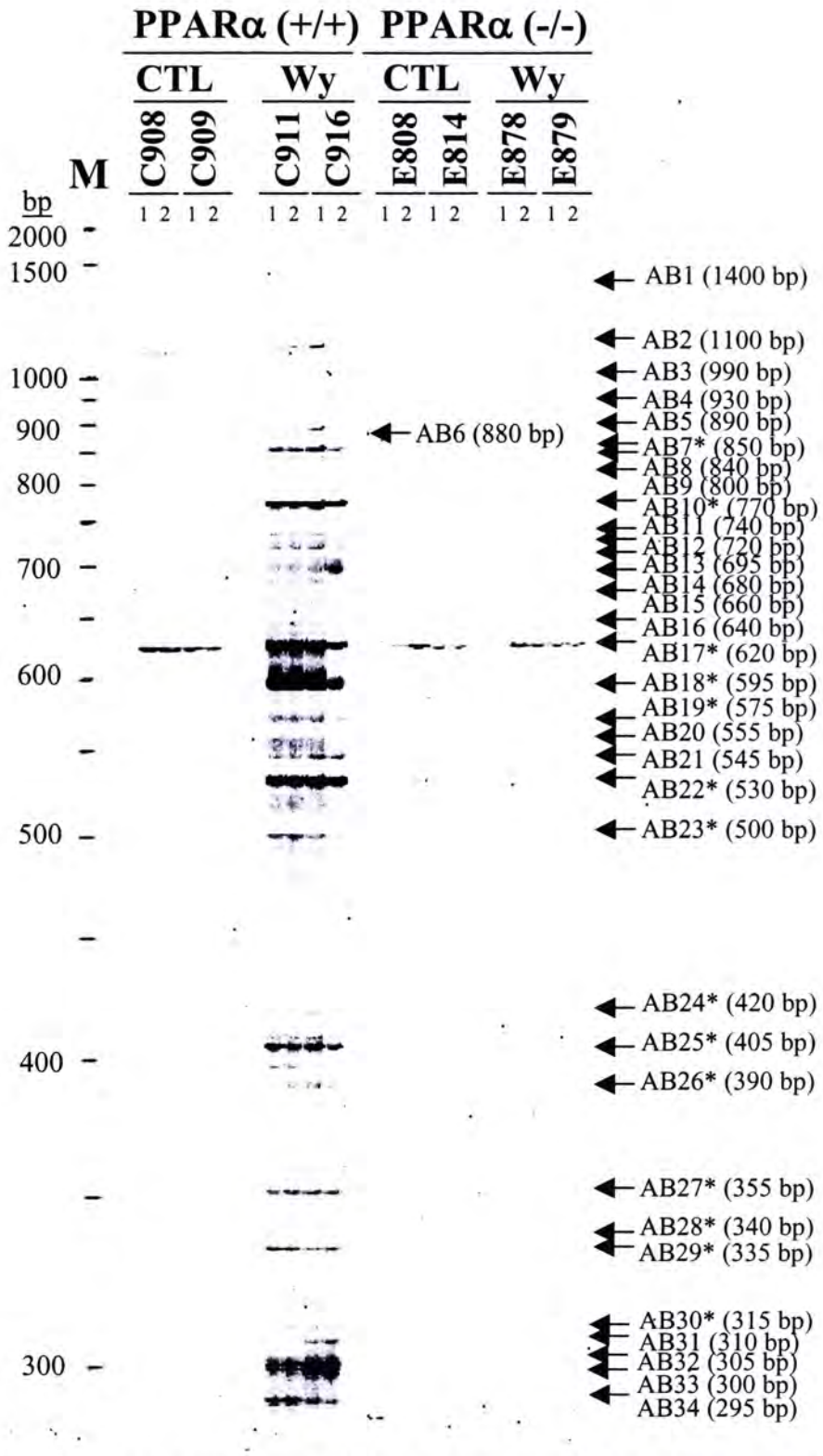

Figure 3.7.2. Fluorescent differential display of gel AB performed with AP3 and ARP3. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP3 and duplicate PCR reactions were performed for each mouse with 3'-TMR-labeled AP3 (ACGACTCACTATAGGGCTTTTTTTTTTTGG) and 5'-ARP3 (ACAATTTCACACAGGAGACCATTGCA). The TMR-labeled fluorescent PCR products were run on a 5.6% denaturing polyacrylamide gel. In gel AB, thirty-four cDNA fragments showing differential display patterns were excised. *Fragments selected for reamplification. M, TMR-labeled molecular weight DNA marker.

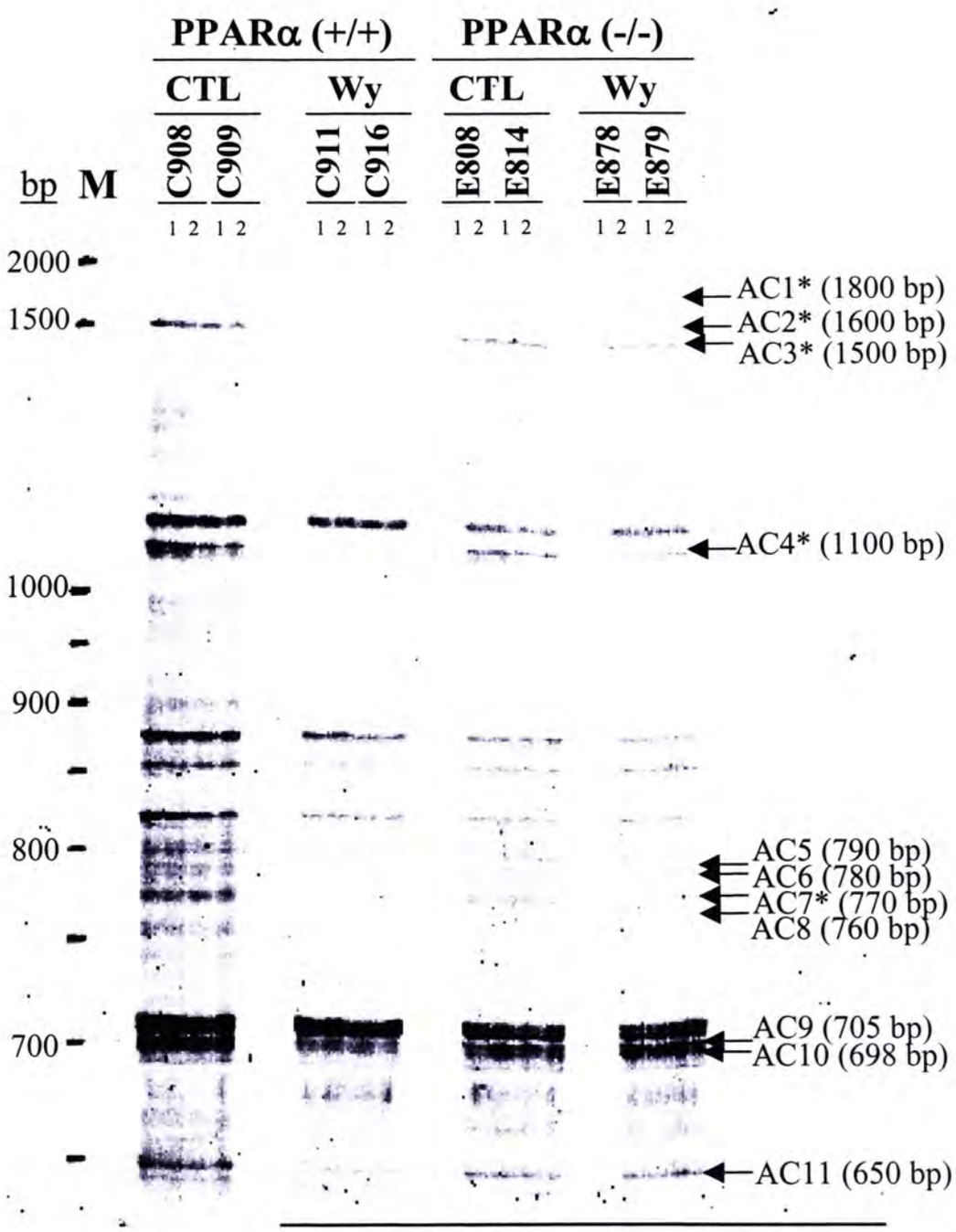

Figure 3.7.3. Fluorescent differential display of gel AC performed with AP2 and ARP19. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP2 and duplicate PCR reactions were performed for each mouse with 3'-TMR-labeled AP2 (ACGACTCACTATAGGGCTTTTTTTTTTTGC) and 5'-ARP19 (ACAATTTCACACAGGATTTTGGCTCC). The TMR-labeled fluorescent PCR products were run on a 5.6% denaturing polyacrylamide gel. In gel AC, eleven cDNA fragments showing differential display patterns were excised. *Fragments selected for reamplification. M, TMR-labeled molecular weight DNA marker.

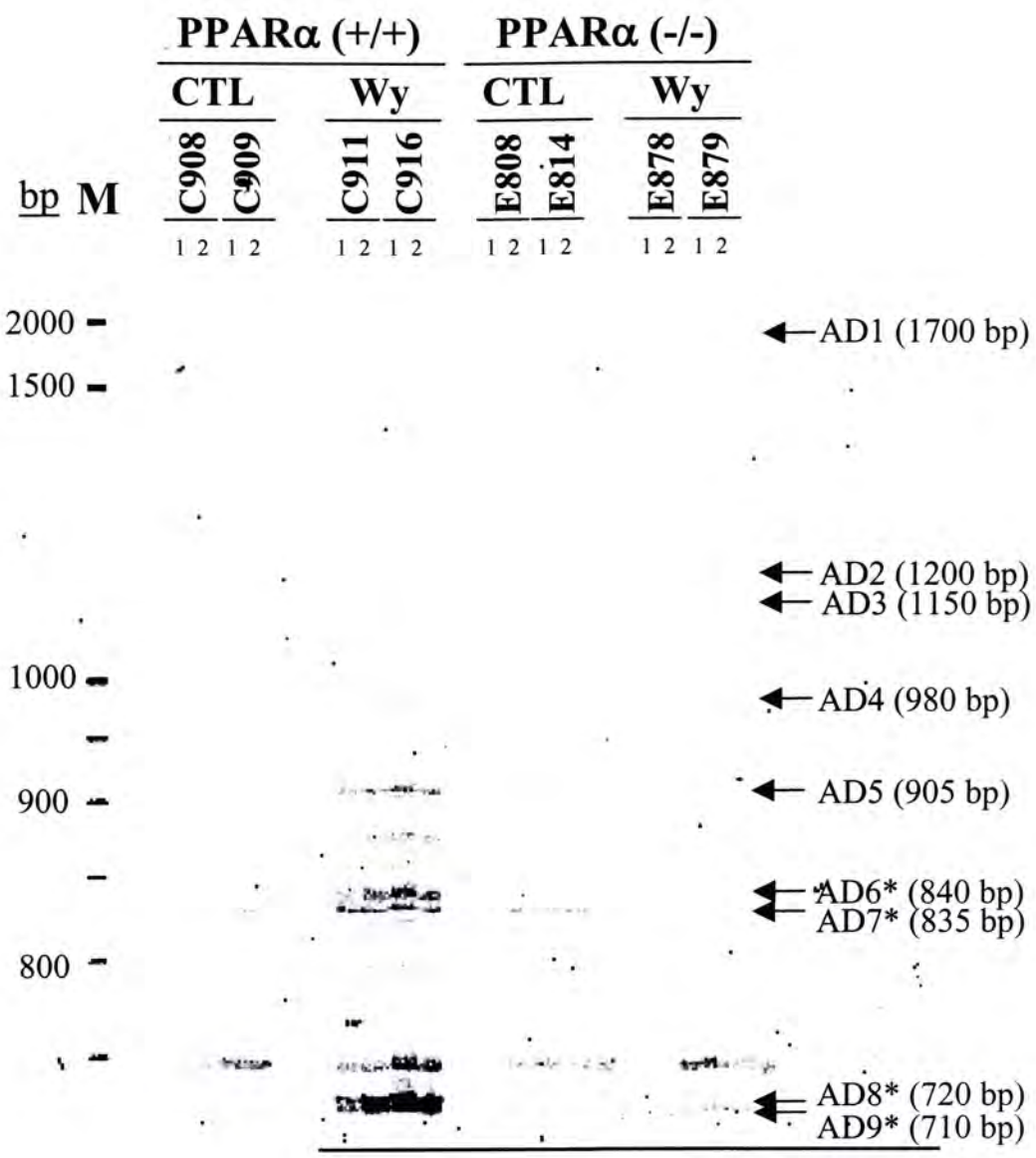

Figure 3.7.4. Fluorescent differential display of gel AD performed with AP2 and ARP18. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP2 and duplicate PCR reactions were performed for each mouse with 3'-TMR-labeled AP2 (ACGACTCACTATAGGGCTTTTTTTTTTTGC) and 5'-ARP18 (ACAATTTCACACAGGATGATGCTACC). The TMR-labeled fluorescent PCR products were run on a 5.6% denaturing polyacrylamide gel. In gel AD, nine cDNA fragments showing differential display patterns were excised. *Fragments selected for reamplification. M, TMR-labeled molecular weight DNA marker.

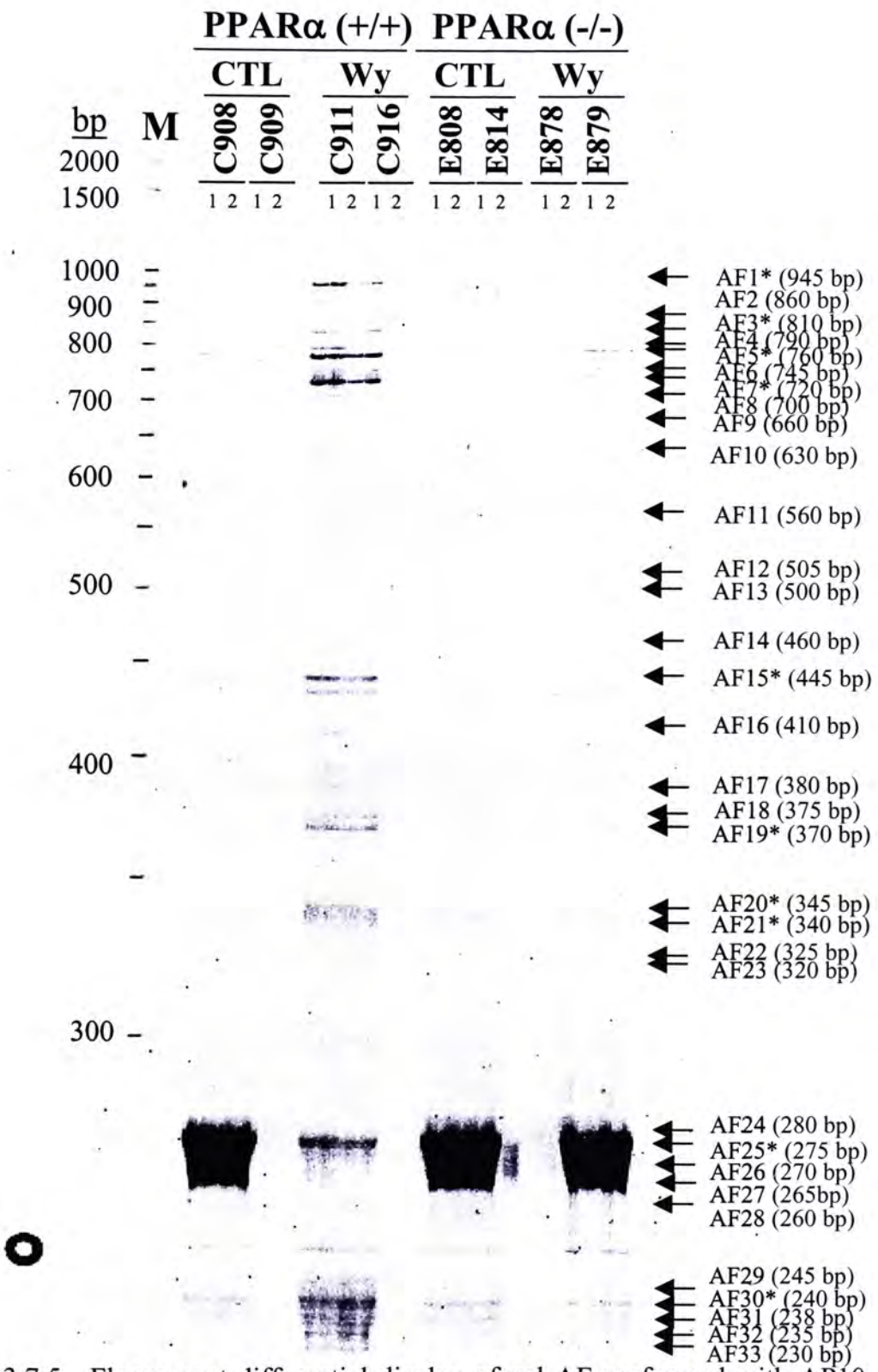

Figure 3.7.5. Fluorescent differential display of gel AF performed with AP10 and ARP13. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP10 and duplicate PCR reactions were performed for each mouse with 3'-TMR-labeled AP10 (ACGACTCACTATAGGGCTTTTTTTTTTTAG) and 5'-ARP13 (ACAATTTCACACAGGAGTTGCACCAT). The TMR-labeled fluorescent PCR products were run on a 5.6% denaturing polyacrylamide gel. In gel AF, thirty-three cDNA fragments showing differential display patterns were excised. *Fragments selected for reamplification. M, TMR-labeled molecular weight DNA marker.

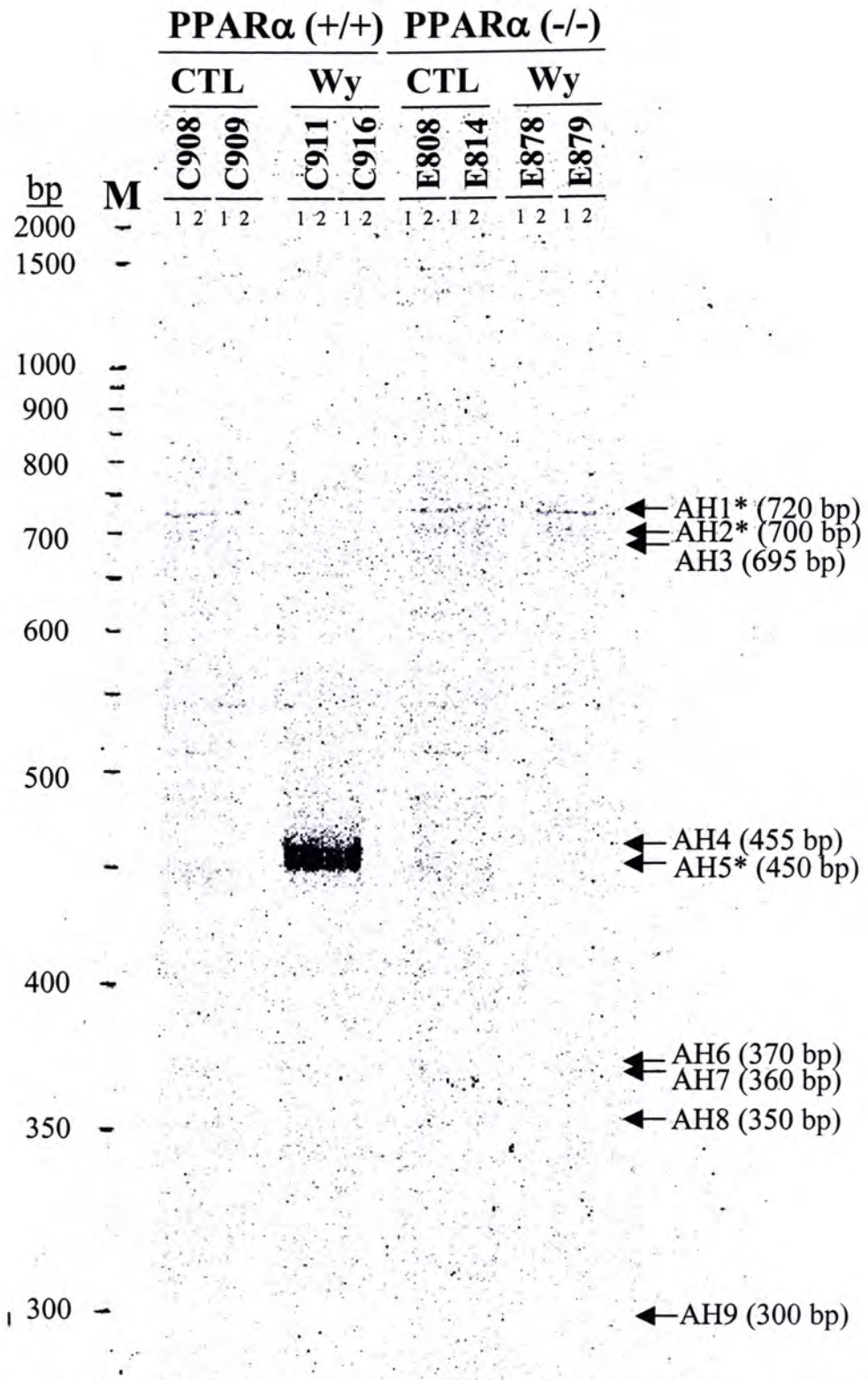

Figure 3.7.6. Fluorescent differential display of gel AH performed with AP11 and ARP19. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP11 and duplicate PCR reactions were performed for each mouse with 3'-TMR-labeled AP11 (ACGACTCACTATAGGGCTTTTTTTTTTTAT) and 5'-ARP19 (ACAATTTCACACAGGATTTTGGCTCC). The TMR-labeled fluorescent PCR products were run on a 5.6% denaturing polyacrylamide gel. In gel AH, nine cDNA fragments showing differential display patterns were excised. *Fragments selected for reamplification. M, TMR-labeled molecular weight DNA marker.

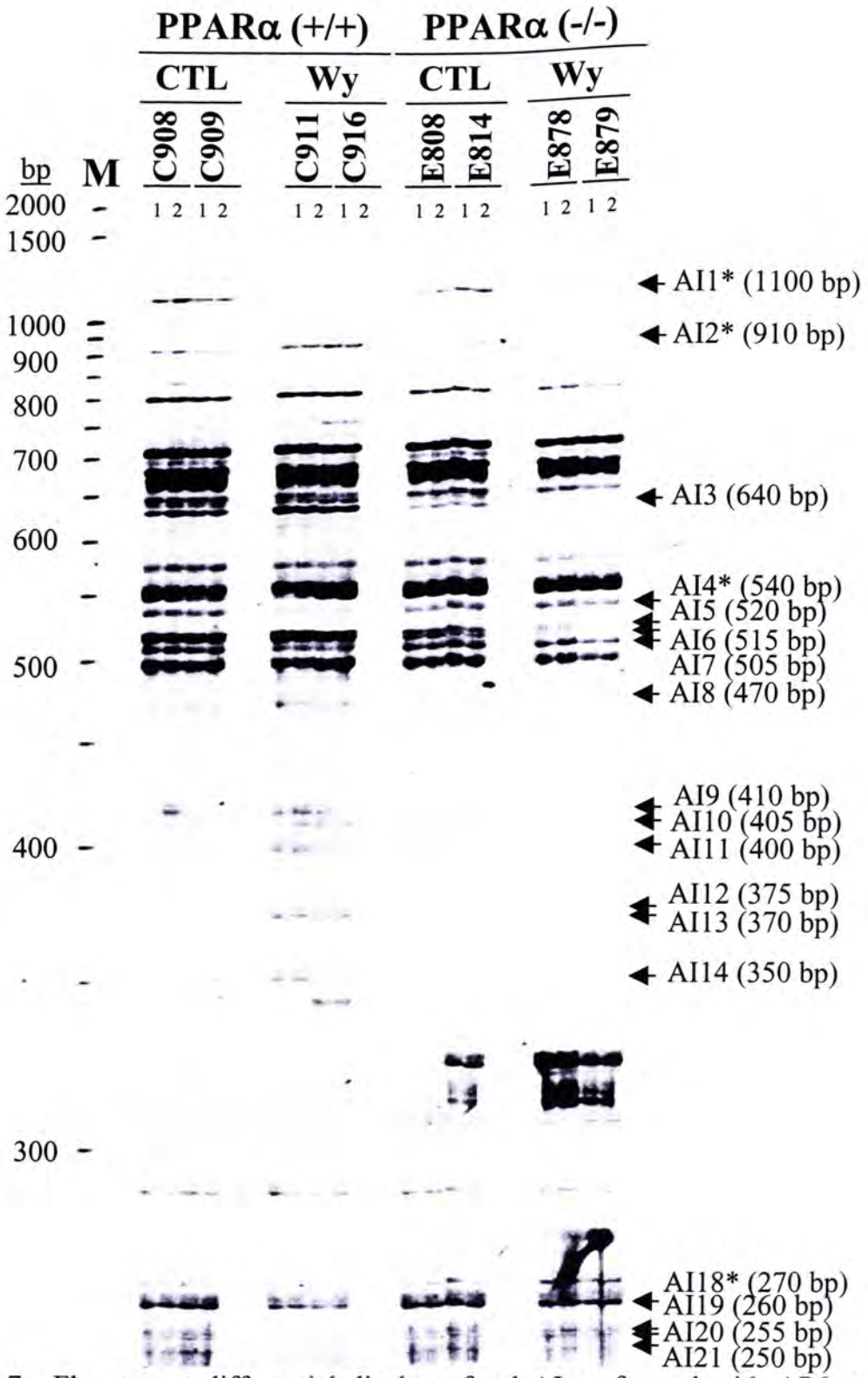

Figure 3.7.7. Fluorescent differential display of gel AI performed with AP6 and ARP4. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP6 and duplicate PCR reactions were performed for each mouse with 3'-TMR-labeled AP6 (ACGACTCACTATAGGGCTTTTTTTTTTTCC) and 5'-ARP4 (ACAATTTCACACAGGAGCTAGCAGAC). The TMR-labeled fluorescent PCR products were run on a 5.6% denaturing polyacrylamide gel. In gel AI, eighteen cDNA fragments showing differential display patterns were excised. *Fragments selected for reamplification. M, TMR-labeled molecular weight DNA marker.

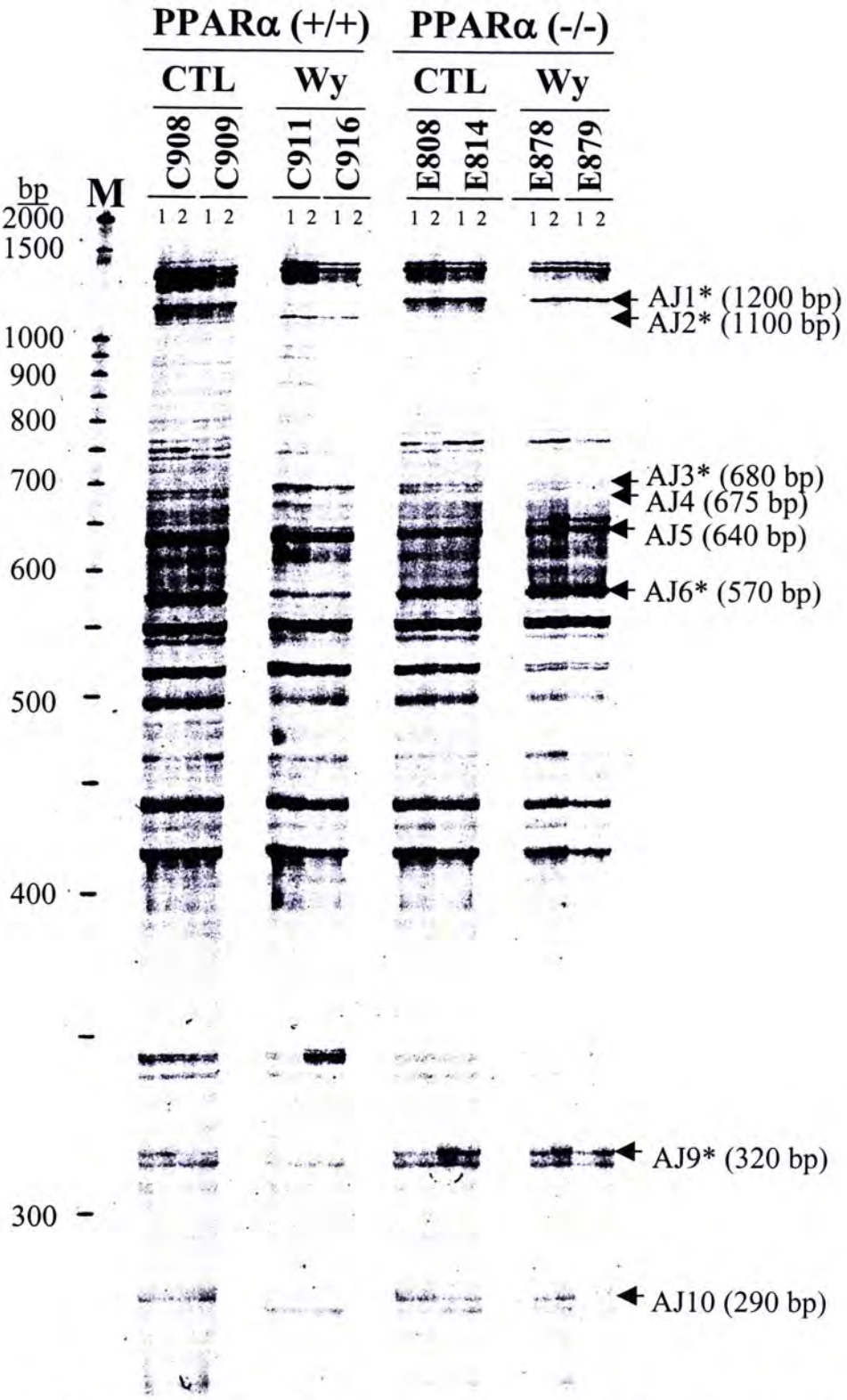

Figure 3.7.8. Fluorescent differential display of gel AJ performed with AP6 and ARP14. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP6 and duplicate PCR reactions were performed for each mouse with 3'-TMR-labeled AP6 (ACGACTCACTATAGGGCTTTTTTTTTTTCC) and 5'-ARP14 (ACAATTTCACACAGGATCCATGACTC). The TMR-labeled fluorescent PCR products were run on a 5.6% denaturing polyacrylamide gel. In gel AJ, eight cDNA fragments showing differential display patterns were excised. *Fragments selected for reamplification. M, TMR-labeled molecular weight DNA marker.

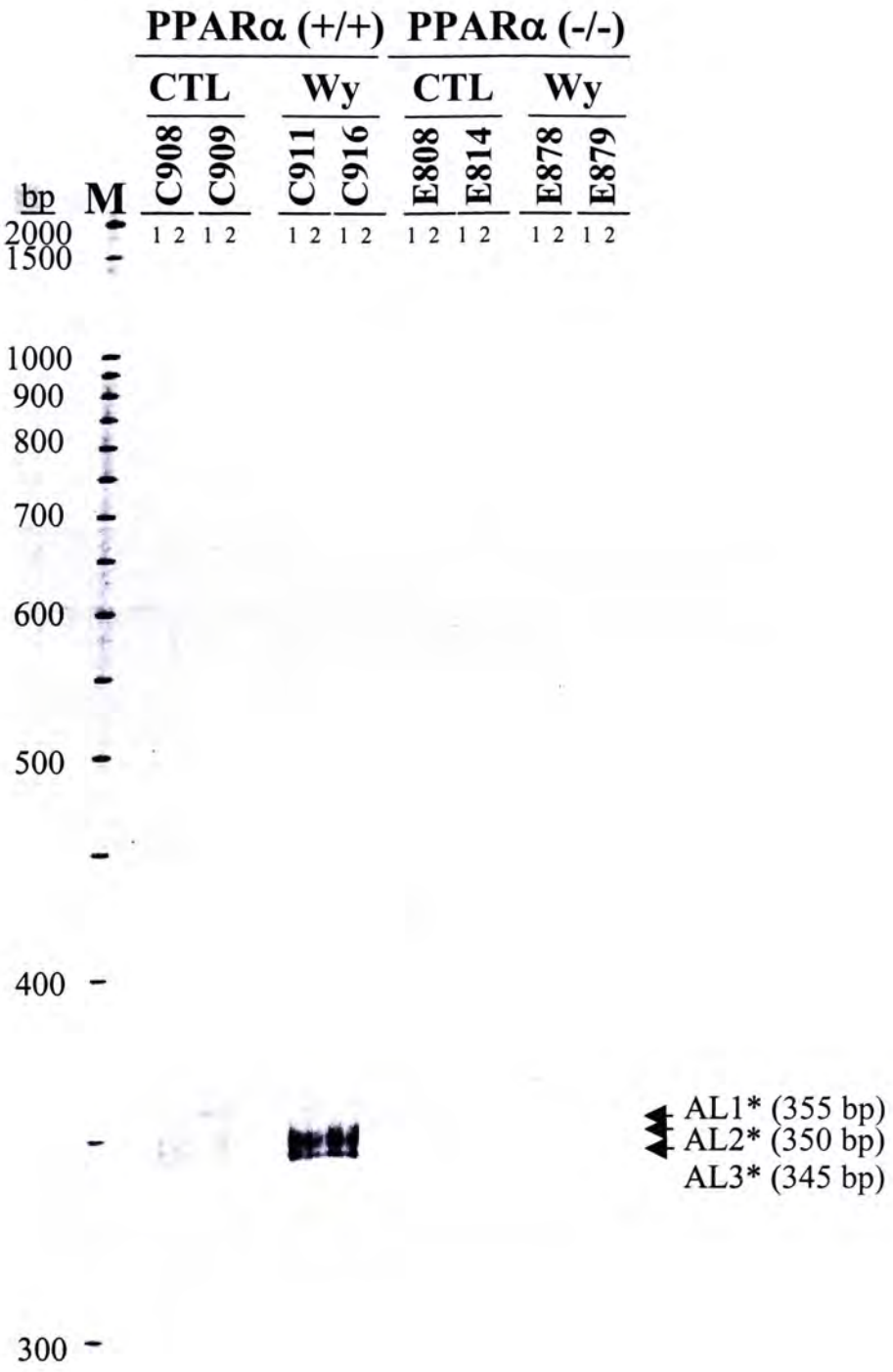

Figure 3.7.9. Fluorescent differential display of gel AL performed with AP7 and ARP15. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP7 and duplicate PCR reactions were performed for each mouse with 3'-TMR-labeled AP7 (ACGACTCACTATAGGGCTTTTTTTTTTTTCG) and 5'-ARP15 (ACAATTTCACACAGGACTTTCTACCC). The TMR-labeled fluorescent PCR products were run on a 5.6% denaturing polyacrylamide gel. In gel AL, three cDNA fragments showing differential display patterns were excised. *Fragments selected for reamplification. M, TMR-labeled molecular weight DNA marker.

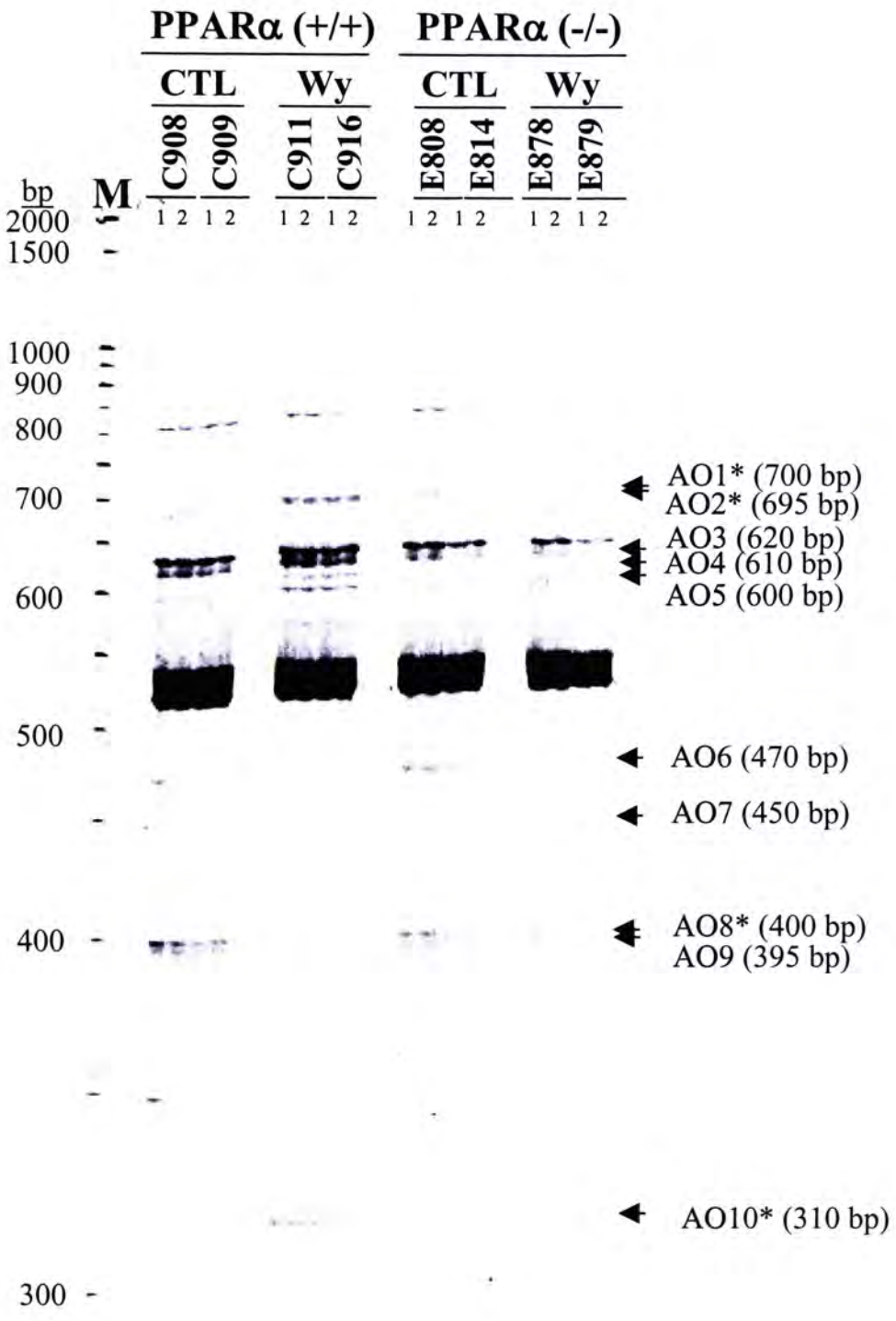

Figure 3.7.10. Fluorescent differential display of gel AO performed with AP5 and ARP10. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP5 and duplicate PCR reactions were performed for each mouse with 3'-TMR-labeled AP5 (ACGACTCACTATAGGGCTTTTTTTTTTTTCA) and 5'-ARP10 (ACAATTTCACACAGGAGATCTCAGAC). The TMR-labeled fluorescent PCR products were run on a 5.6% denaturing polyacrylamide gel. In gel AO, ten cDNA fragments showing differential display patterns were excised. *Fragments selected for reamplification. M, TMR-labeled molecular weight DNA marker.

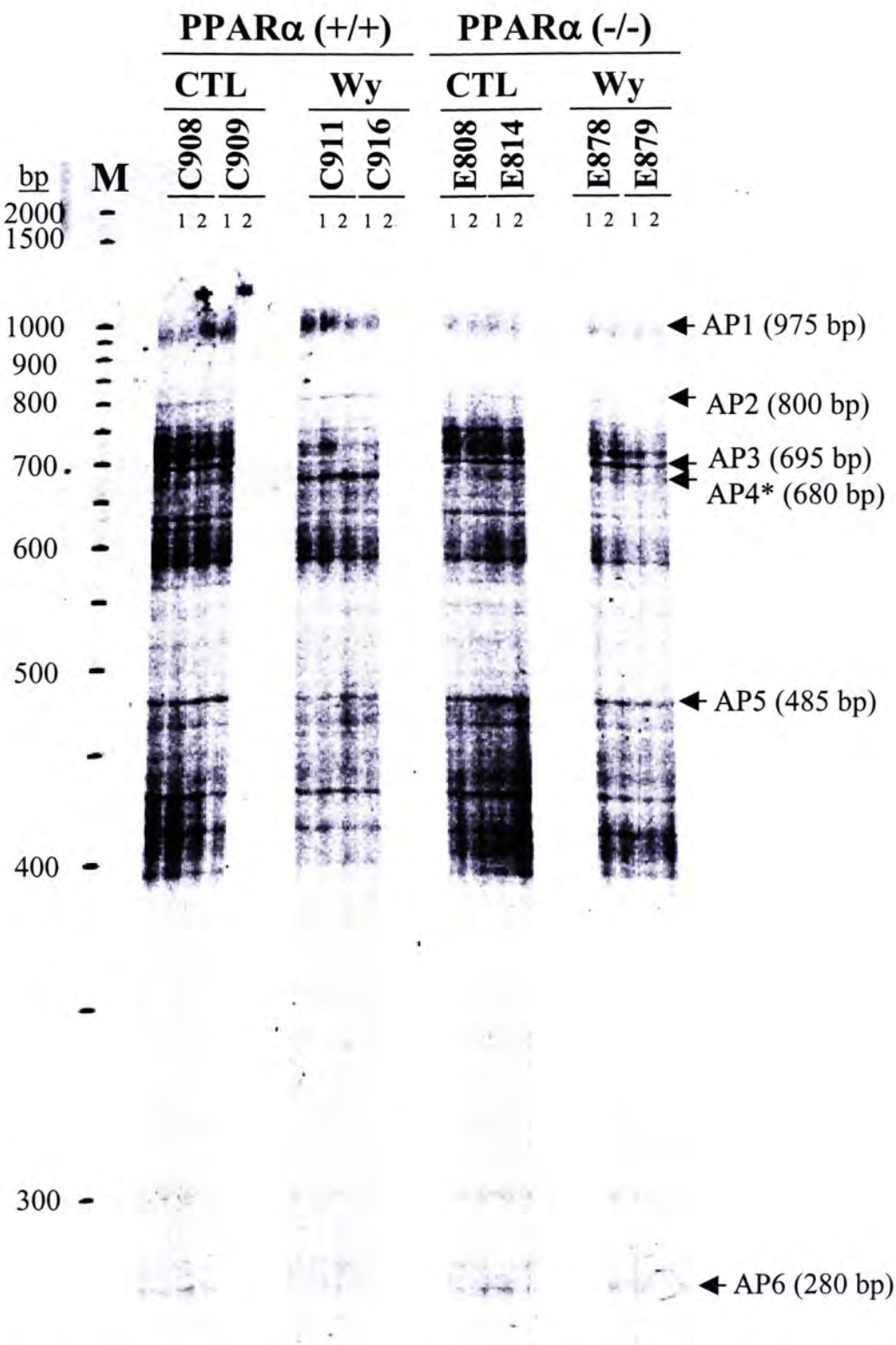

Figure 3.7.11. Fluorescent differential display of gel AP performed with AP12 and ARP2. Total RNA from PPARα (+/+) and PPARα (-/-) mouse livers treated with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was reverse transcribed with AP12 and duplicate PCR reactions were performed for each mouse with 3'-TMR-labeled AP12 (ACGACTCACTATAGGGCTTTTTTTTTTTCT) and 5'-ARP2 (ACAATTTCACACAGGAGCTAGCATGG). The TMR-labeled fluorescent PCR products were run on a 5.6% denaturing polyacrylamide gel. In gel AP, six cDNA fragments showing differential display patterns were excised. *Fragments selected for reamplification. M, TMR-labeled molecular weight DNA marker.

Table 3.7.1. Summary of cDNA fragments excised from gel AA (AP1 and ARP2)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Differential display pattern					
			PPARα (+/+)		PPARα (-/-)		FluoroDD pattern§	PPARα-dependent genes that were either up- or down-regulated in PPARα (+/+) mice treated with 0.1% Wy-14,643 compared to their corresponding controls
			CTL	Wy	CTL	Wy		
AA (AP1 & ARP2)	AA1*	1400	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AA2*	1300	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AA3*	750	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AA4*	695	++	+	++	++	IV	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AA5*	670	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AA6*	660	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AA7*	640	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AA8*	570	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AA9*	520	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AA10*	500	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AA11	490	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AA12*	470	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AA14	395	++	+	++	++	IV	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AA15	390	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AA16	385	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AA17	345	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent

'+' represents the presence of a band (cDNA) on fluoroDD gel, while '-' represents the corresponding band (cDNA) that was not observed on the fluoroDD gel. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. *Fragments selected for reamplification. §Differential expression patterns as shown in Table 4.1.

AB17, AB18, AB19, AB20, AB21, AB22, AB23, AB24, AB25, AB26, AB27, AB28, AB29, AB30, AB31, AB32, AB33 and AB34) (Figure 3.7.2) (Tables 3.7.2-3.7.3). For gel AC, eleven bands were excised (AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8, AC9, AC10 and AC11) (Figure 3.7.3) (Table 3.7.4). For gel AD, nine bands were excised (AD1, AD2, AD3, AD4, AD5, AD6, AD7, AD8 and AD9) (Figure 3.7.4) (Table 3.7.5). For gel AF, thirty-three bands were excised (AF1, AF2, AF3, AF4, AF5, AF6, AF7, AF8, AF9 AF10, AF11, AF12, AF13, AF14, AF15, AF16, AF17, AF18, AF19, AF20, AF21, AF22, AF23, AF24, AF25, AF26, AF27, AF28, AF29, AF30, AF31, AF32 and AF33) (Figure 3.7.5) (Tables 3.7.6-3.7.7). Nine fragments were excised from gel AH (AH1, AH2, AH3, AH4, AH5, AH6, AH7, AH8 and AH9) (Figure 3.7.6) (Table 3.7.8). For gel AI, eighteen fragments were selected for excision (AI1, AI2, AI3, AI4, AI5, AI6, AI7, AI8, AI9, AI10, AI11, AI12, AI13, AI14, AI18, AI19, AI20 and AI21) (Figure 3.7.7) (Table 3.7.9). For gel AJ, eight fragments were selected for excision (AJ1, AJ2, AJ3, AJ4, AJ5, AJ6, AJ9 and AJ10) (Figure 3.7.8) (Table 3.7.10). For gel AL, three fragments were excised (AL1, AL2 and AL3) (Figure 3.7.9) (Table 3.7.10). Ten fragments were excised from gel AO (AO1, AO2, AO3, AO4, AO5, AO6, AO7, AO8, AO9 and AO10) (Figure 3.7.10) (Table 3.7.11). For gel AP, six fragments were excised (AP1, AP2, AP3, AP4, AP5 and AP6) (Figure 3.7.11) (Table 3.7.11).

3.8 Reamplification of fluorescent differential display (fluoroDD) fragments

The cDNA fragments were reamplified with full-length M13 reverse (-48) 24-mer (5'-AGCGGATAACAATTTCACACAGGA-3') and T7 promoter 22-mer (5'-

Table 3.7.2. Summary of cDNA fragments excised from gel AB (AP3 and ARP3) Part I

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Differential display pattern					
			PPARα (+/+)		PPARα (-/-)		FluoroDD pattern§	PPARα-dependent genes that were either up- or down-regulated in PPARα (+/+) mice treated with 0.1% Wy-14,643 compared to their corresponding controls
			CTL	Wy	CTL	Wy		
AB (AP3 & ARP3)	AB1	1400	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB2	1100	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB3	990	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB4	930	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB5	890	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB6	880	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB7*	850	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB8	840	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB9	800	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB10*	770	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB11	740	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB12	720	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB13	695	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB14	680	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB15	660	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB16	640	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB17*	620	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent

'+' represents the presence of a band (cDNA) on fluoroDD gel, while '-' represents the corresponding band (cDNA) that was not observed on the fluoroDD gel. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. *Fragments selected for reamplification. §Differential expression patterns as shown in Table 4.1.

Table 3.7.3. Summary of cDNA fragments excised from gel AB (AP3 and ARP3) Part II

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Differential display pattern					
			PPARα (+/+)		PPARα (-/-)		FluoroDD pattern[§]	PPARα-dependent genes that were either up- or down-regulated in PPARα (+/+) mice treated with 0.1% Wy-14,643 compared to their corresponding controls
			CTL	Wy	CTL	Wy		
AB (AP3 & ARP3)	AB18*	595	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB19*	575	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB20	555	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB21	545	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB22*	530	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB23*	500	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB24*	420	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB25*	405	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB26*	390	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB27*	355	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB28*	340	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB29*	335	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB30*	315	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB31	310	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB32	305	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB33	300	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AB34	295	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent

'+' represents the presence of a band (cDNA) on fluoroDD gel, while '-' represents the corresponding band (cDNA) that was not observed on the fluoroDD gel. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. *Fragments selected for reamplification. [§]Differential expression patterns as shown in Table 4.1.

Table 3.7.4. Summary of cDNA fragments excised from gel AC (AP2 and ARP19)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Differential display pattern					
			PPARα (+/+)		PPARα (-/-)		FluoroDD pattern[§]	PPARα-dependent genes that were either up- or down-regulated in PPARα (+/+) mice treated with 0.1% Wy-14,643 compared to their corresponding controls
			CTL	Wy	CTL	Wy		
AC (AP2 & ARP19)	AC1*	1800	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AC2*	1600	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AC3*	1500	++	+	++	++	IV	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AC4*	1100	++	+	++	++	IV	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AC5	790	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AC6	780	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AC7*	770	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AC8	760	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AC9	705	++	+	++	++	IV	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AC10	698	++	+	++	++	IV	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AC11	650	++	+	++	++	IV	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent

'+' represents the presence of a band (cDNA) on fluoroDD gel, while '-' represents the corresponding band (cDNA) that was not observed on the fluoroDD gel. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. *Fragments selected for reamplification. [§]Differential expression patterns as shown in Table 4.1.

Table 3.7.5. Summary of cDNA fragments excised from gel AD (AP2 and ARP18)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Differential display pattern					
			PPARα (+/+)		PPARα (-/-)		FluoroDD pattern[§]	PPARα-dependent genes that were either up- or down-regulated in PPARα (+/+) mice treated with 0.1% Wy-14,643 compared to their corresponding controls
			CTL	Wy	CTL	Wy		
AD (AP2 & ARP18)	AD1	1700	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AD2	1200	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AD3	1150	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AD4	980	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AD5	905	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AD6*	840	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AD7*	835	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AD8*	720	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AD9*	710	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent

'+' represents the presence of a band (cDNA) on fluoroDD gel, while '-' represents the corresponding band (cDNA) that was not observed on the fluoroDD gel. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. *Fragments selected for reamplification. [§]Differential expression patterns as shown in Table 4.1.

Table 3.7.6. Summary of cDNA fragments excised from gel AF (AP10 and ARP13) Part I

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Differential display pattern					
			PPARα (+/+)		PPARα (-/-)		FluoroDD pattern[§]	PPARα-dependent genes that were either up- or down-regulated in PPARα (+/+) mice treated with 0.1% Wy-14,643 compared to their corresponding controls
			CTL	Wy	CTL	Wy		
AF (AP10 & ARP13)	AF1*	945	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF2	860	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF3*	810	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF4	790	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF5*	760	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF6	745	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF7*	720	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF8	700	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF9	660	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF10	630	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF11	560	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF12	505	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF13	500	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF14	460	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF15*	445	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF16	410	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF17	380	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent

'+' represents the presence of a band (cDNA) on fluoroDD gel, while '-' represents the corresponding band (cDNA) that was not observed on the fluoroDD gel. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. *Fragments selected for reamplification. [§]Differential expression patterns as shown in Table 4.1.

Table 3.7.7. Summary of cDNA fragments excised from gel AF (AP10 and ARP13) Part II

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Differential display pattern					
			PPARα (+/+)		PPARα (-/-)		FluoroDD pattern[§]	PPARα-dependent genes that were either up- or down-regulated in PPARα (+/+) mice treated with 0.1% Wy-14,643 compared to their corresponding controls
			CTL	Wy	CTL	Wy		
AF (AP10 & ARP13)	AF18	375	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF19*	370	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF20*	345	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF21*	340	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF22	325	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF23	320	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF24	280	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF25*	275	++	+	++	++	IV	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF26	270	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF27	265	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF28	260	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF29	245	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF30*	240	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF31	238	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF32	235	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AF33	230	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent

'+' represents the presence of a band (cDNA) on fluoroDD gel, while '-' represents the corresponding band (cDNA) that was not observed on the fluoroDD gel. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. *Fragments selected for reamplification. [§]Differential expression patterns as shown in Table 4.1.

Table 3.7.8. Summary of cDNA fragments excised from gel AH (AP11 and ARP19)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Differential display pattern					
			PPARα (+/+)		PPARα (-/-)		FluoroDD pattern[§]	PPARα-dependent genes that were either up- or down-regulated in PPARα (+/+) mice treated with 0.1% Wy-14,643 compared to their corresponding controls
			CTL	Wy	CTL	Wy		
AH (AP11 & ARP19)	AH1*	720	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AH2*	700	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AH3	695	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AH4	455	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AH5*	450	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AH6	370	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AH7	360	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AH8	350	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AH9	300	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent

'+' represents the presence of a band (cDNA) on fluoroDD gel, while '-' represents the corresponding band (cDNA) that was not observed on the fluoroDD gel. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. *Fragments selected for reamplification. [§]Differential expression patterns as shown in Table 4.1.

Table 3.7.9. Summary of cDNA fragments excised from gel AI (AP6 and ARP4)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Differential display pattern					
			PPARα (+/+)		PPARα (-/-)		FluoroDD pattern[§]	PPARα-dependent genes that were either up- or down-regulated in PPARα (+/+) mice treated with 0.1% Wy-14,643 compared to their corresponding controls
			CTL	Wy	CTL	Wy		
AI (AP6 & ARP4)	AI1*	1100	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI2*	910	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI3	640	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI4*	540	++	+	++	++	IV	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI5	520	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI6	515	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI7	505	++	+	++	++	IV	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI8	470	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI9	410	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI10	405	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI11	400	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI12	375	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI13	370	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI14	350	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI18*	270	++	+	++	++	IV	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI19	260	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI20	255	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AI21	250	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent

'+' represents the presence of a band (cDNA) on fluoroDD gel, while '-' represents the corresponding band (cDNA) that was not observed on the fluoroDD gel. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. *Fragments selected for reamplification. [§]Differential expression patterns as shown in Table 4.1.

Table 3.7.10. Summary of cDNA fragments excised from gels AJ (AP6 and ARP14) and AL (AP7 and ARP15)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Differential display pattern					
			PPARα (+/+)		PPARα (-/-)		FluoroDD pattern[§]	PPARα-dependent genes that were either up- or down-regulated in PPARα (+/+) mice treated with 0.1% Wy-14,643 compared to their corresponding controls
			CTL	Wy	CTL	Wy		
AJ (AP6 & ARP14)	AJ1*	1200	++	+	++	++	IV	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AJ2*	1100	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AJ3*	680	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AJ4	675	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AJ5	640	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AJ6*	570	++	+	++	++	IV	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AJ9*	320	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AJ10	290	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
AL (AP7 & ARP15)	AL1*	355	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AL2*	350	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AL3*	345	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent

'+' represents the presence of a band (cDNA) on fluoroDD gel, while '-' represents the corresponding band (cDNA) that was not observed on the fluoroDD gel. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. *Fragments selected for reamplification. [§]Differential expression patterns as shown in Table 4.1.

Table 3.7.11. Summary of cDNA fragments excised from gels AO (AP5 and ARP10) and AP (AP12 and ARP2)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Differential display pattern					
			PPARα (+/+)		PPARα (-/-)		FluoroDD pattern§	PPARα-dependent genes that were either up- or down-regulated in PPARα (+/+) mice treated with 0.1% Wy-14,643 compared to their corresponding controls
			CTL	Wy	CTL	Wy		
AO (AP5 & ARP10)	AO1*	700	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AO2*	695	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AO3	620	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AO4	610	-	+	-	-	I	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AO5	600	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AO6	470	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AO7	450	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AO8*	400	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AO9	395	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AO10*	310	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
AP (AP12 & ARP2)	AP1	975	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AP2	800	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AP3	695	++	+	++	++	IV	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AP4*	680	+	++	+	+	II	Up-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AP5	485	++	+	++	++	IV	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent
	AP6	280	+	-	+	+	III	Down-regulated in 0.1% Wy-14,643 treatment and PPARα dependent

'+' represents the presence of a band (cDNA) on fluoroDD gel, while '-' represents the corresponding band (cDNA) that was not observed on the fluoroDD gel. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. *Fragments selected for reamplification. §Differential expression patterns as shown in Table 4.1.

GTAATACGACTCACTATAGGGC-3') primers. As partial sequences of M13 reverse (16-mer) and T7 promoter (17-mer) were present in the 5'-ARP and 3'-AP primers of double-strand cDNA synthesis, respectively, the reamplified PCR products were lengthened by 8 nucleotides from the M13 reverse primer and 5 nucleotides from the T7 promoter primer. The expected size of reamplified PCR product was 13 nucleotides longer than the size of fluoroDD fragments. A total of sixty-four (41%) cDNA fragments were chosen for reamplification [AA1, AA2, AA3, AA4, AA5, AA6, AA7, AA8, AA9, AA10 and AA12 (Figure 3.8.1) (Table 3.8.1); AB7, AB10, AB17, AB18, AB19, AB22, AB23, AB24, AB25, AB26, AB27, AB28, AB29 and AB30 (Figures 3.8.2-3.8.3) (Table 3.8.2); AC1, AC2, AC3, AC4, AC7, AD6, AD7, AD8, AD9, AF1, AF3, AF5, AF7, AF15, AF19, AF20, AF21, AF25 and AF30 (Figures 3.8.4-3.5.5) (Table 3.8.3); AH1, AH2, AH5, AI1, AI2, AI4, AI18, AJ1, AJ2, AJ3, AJ6, AJ9, AL1, AL2, AL3, AO1, AO2, AO8, AO10 and AP4 (Figures 3.8.6-3.8.7) (Tables 3.8.4-3.8.5)]. The sizes of fragments AB19 and AB26 (Table 3.8.2); AD6 and AF30 (Table 3.8.3); AH2 (Table 3.8.4); and AL3 (Table 3.8.5) were bigger than the expected sizes, while the fragment sizes of AA2 (Table 3.8.1); AB10 (Table 3.8.2); AC7 (Table 3.8.3); and AO1 (Table 3.8.5) were smaller than the expected sizes. These might be due to the contamination during band excision. cDNA fragments AA1 and AA5 (Table 3.8.1); AB23 (Table 3.8.2); AC2 and AF7 (Table 3.8.3); and AJ6 (Table 3.8.4) contained extra PCR products, which might be due to contamination of co-migration and/or nearby cDNA fragments during bands excision. All the reamplified fragments with expected sizes were selected for subcloning.

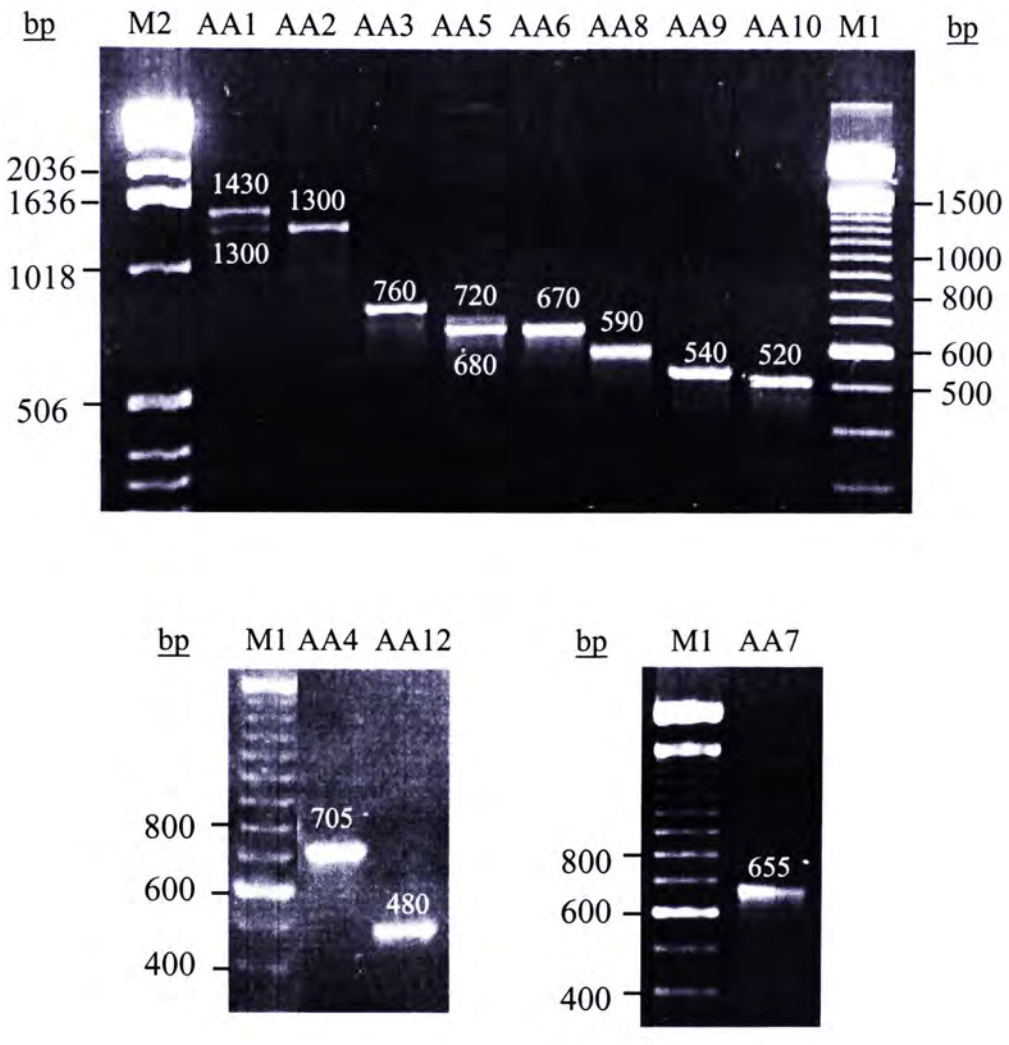

Figure 3.8.1. Reamplification of cDNA fragments excised from fluoroDD gel AA (AP1 and ARP2). The cDNA fragments excised from the gel were amplified by PCR using full-length M13 reverse (-48) 24-mer (5'-AGCGGATAACAATTTCACACAGGA-3') and T7 promoter 22-mer (5'-GTAATACGACTCACTATAGGGC-3') primers. The PCR products were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. M1, 100 bp DNA marker; M2, 1 kb DNA marker.

Table 3.8.1. Summary of reamplified cDNA fragments excised from gel AA (AP1 and ARP2)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Differential display pattern				Reamplified fragment size (bp)
			PPARα (+/+)		PPARα (-/-)		
			CTL	Wy	CTL	Wy	
AA (AP1 & ARP2)	AA1	1400	-	+	-	-	1430+1300
	AA2	1300	+	++	+	+	1300
	AA3	750	+	++	+	+	760
	AA4	695	++	+	++	++	705
	AA5	670	+	++	+	+	720+680
	AA6	660	+	++	+	+	670
	AA7	640	+	++	+	+	655
	AA8	570	+	++	+	+	590
	AA9	520	+	++	+	+	540
	AA10	500	+	++	+	+	520
	AA12	470	+	++	+	+	480

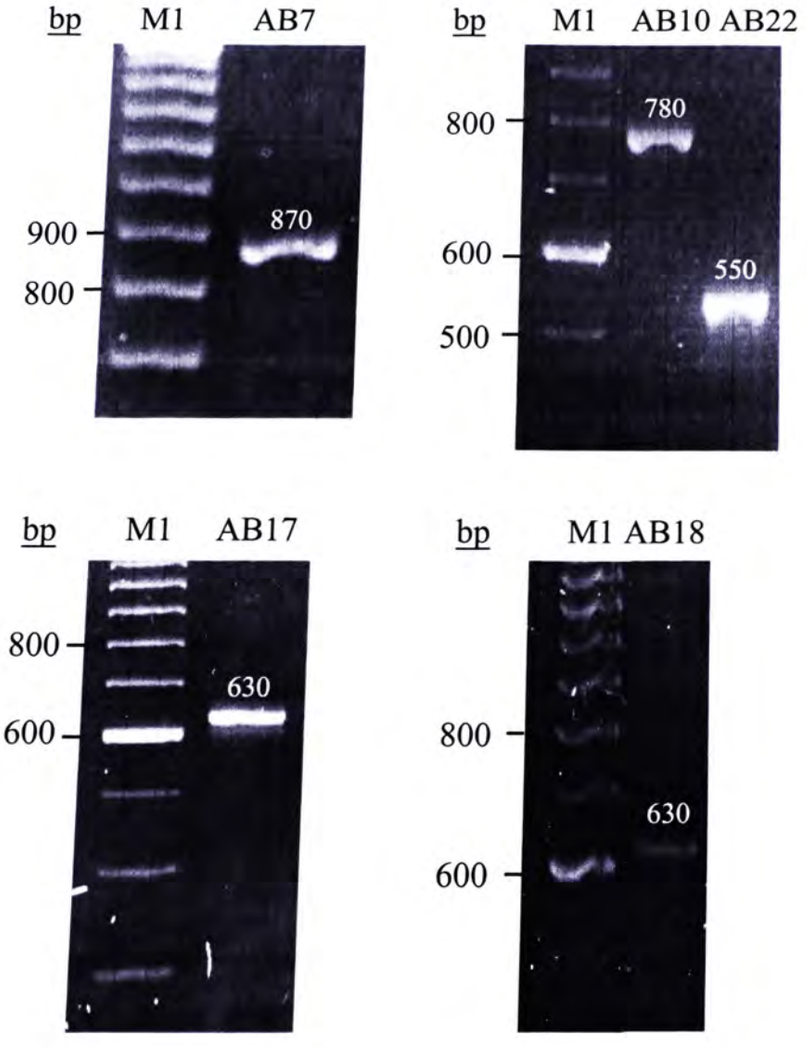

Figure 3.8.2. Reamplification of cDNA fragments excised from fluoroDD gel AB (AP3 and ARP3) Part I. The cDNA fragments excised from the gel were amplified by PCR using full-length M13 reverse (-48) 24-mer (5'-AGCGGATAACAATTTCACACAGGA-3') and T7 promoter 22-mer (5'-GTAATACGACTCACTATAGGGC-3') primers. The PCR products were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. M1, 100 bp DNA marker.

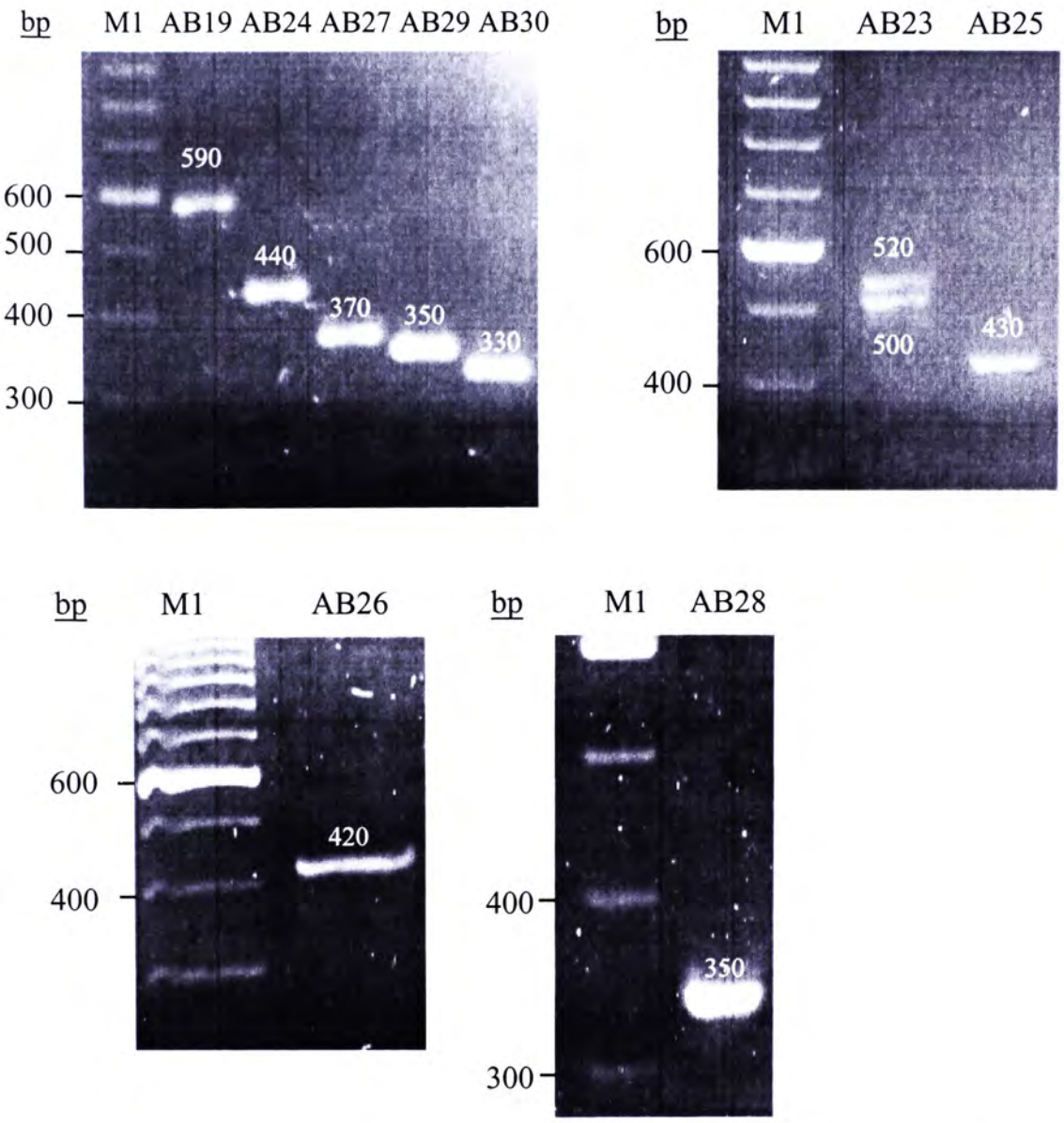

Figure 3.8.3. Reamplification of cDNA fragments excised from fluoroDD gel AB (AP3 and ARP3) Part II. The cDNA fragments excised from the gel were amplified by PCR using full-length M13 reverse (-48) 24-mer (5'-AGCGGATAACAATTTCACACAGGA-3') and T7 promoter 22-mer (5'-GTAATACGACTCACTATAGGGC-3') primers. The PCR products were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. M1, 100 bp DNA marker.

Table 3.8.2. Summary of reamplified cDNA fragments excised from gel AB (AP3 and ARP3)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Differential display pattern				Reamplified fragment size (bp)
			PPARα (+/+)		PPARα (-/-)		
			CTL	Wy	CTL	Wy	
AB (AP3 & ARP3)	AB7	850	+	++	+	+	870
	AB10	770	+	++	+	+	780
	AB17	620	+	++	+	+	630
	AB18	595	+	++	+	+	630
	AB19	575	-	+	-	-	590
	AB22	530	+	++	+	+	550
	AB23	500	-	+	-	-	500+520
	AB24	420	-	+	-	-	440
	AB25	405	-	+	-	-	430
	AB26	390	-	+	-	-	420
	AB27	355	-	+	-	-	370
	AB28	340	-	+	-	-	350
	AB29	335	-	+	-	-	350
	AB30	315	-	+	-	-	330

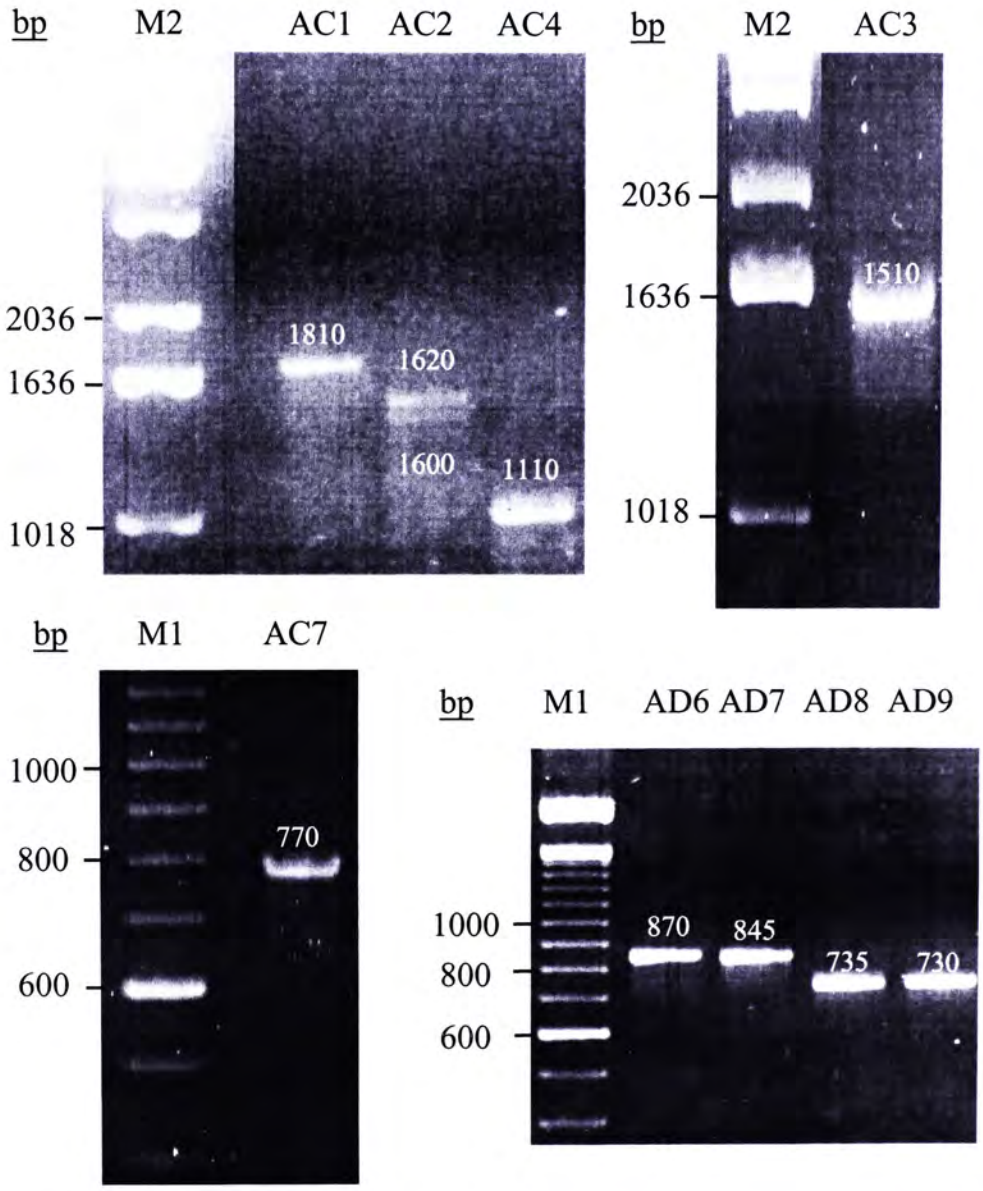

Figure 3.8.4. Reamplification of cDNA fragments excised from fluoroDD gels AC (AP2 and ARP19) and AD (AP2 and ARP18). The cDNA fragments excised from the gel were amplified by PCR using full-length M13 reverse (-48) 24-mer (5'-AGCGGATAACAATTTCACACAGGA-3') and T7 promoter 22-mer (5'-GTAATACGACTCACTATAGGGC-3') primers. The PCR products were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. M1, 100 bp DNA marker; M2, 1 kb DNA marker.

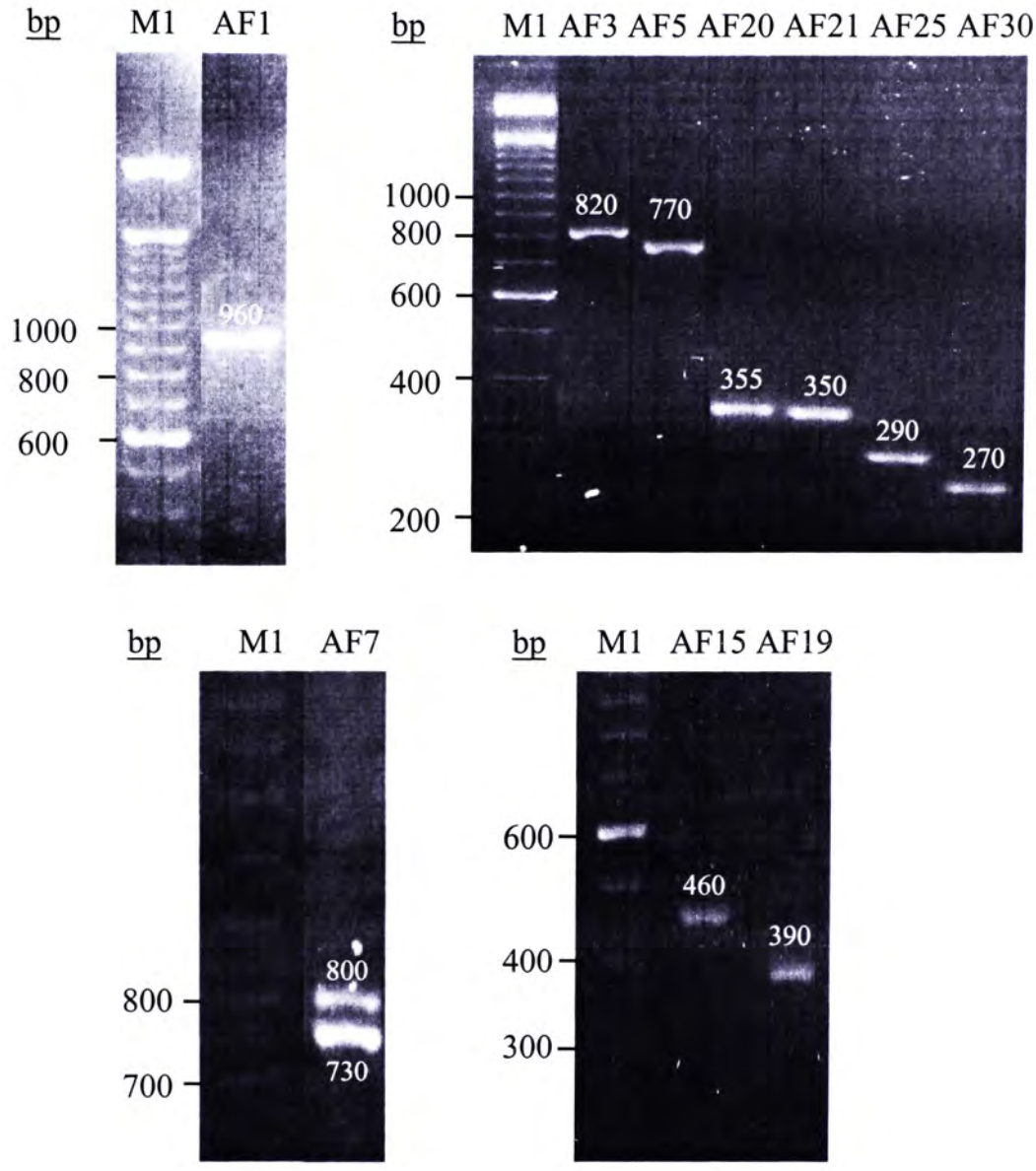

Figure 3.8.5. Reamplification of cDNA fragments excised from fluoroDD gel AF (AP10 and ARP13). The cDNA fragments excised from the gel were amplified by PCR using full-length M13 reverse (-48) 24-mer (5'-AGCGGATAACAATTTCACACAGGA-3') and T7 promoter 22-mer (5'-GTAATACGACTCACTATAGGGC-3') primers. The PCR products were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. M1, 100 bp DNA marker.

Table 3.8.3. Summary of reamplified cDNA fragments excised from gels AC (AP2 and ARP19), AD (AP2 and ARP18) and AF (AP10 and ARP13)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Differential display pattern				Reamplified fragment size (bp)
			PPARα (+/+)		PPARα (-/-)		
			CTL	Wy	CTL	Wy	
AC (AP2 & ARP19)	AC1	1800	+	-	+	+	1810
	AC2	1600	-	+	-	-	1620 + 1600
	AC3	1500	++	+	++	++	1510
	AC4	1100	++	+	++	++	1110
	AC7	770	+	-	+	+	770
AD (AP2 & ARP18)	AD6	840	+	++	+	+	870
	AD7	835	+	++	+	+	845
	AD8	720	+	++	+	+	735
	AD9	710	+	++	+	+	730
AF (AP10 & ARP13)	AF1	945	+	++	+	+	960
	AF3	810	+	++	+	+	820
	AF5	760	+	++	+	+	770
	AF7	720	+	++	+	+	730+800
	AF15	445	+	++	+	+	460
	AF19	370	+	++	+	+	390
	AF20	345	+	++	+	+	355
	AF21	340	+	++	+	+	350
	AF25	275	++	+	++	++	290
	AF30	240	+	++	+	+	270

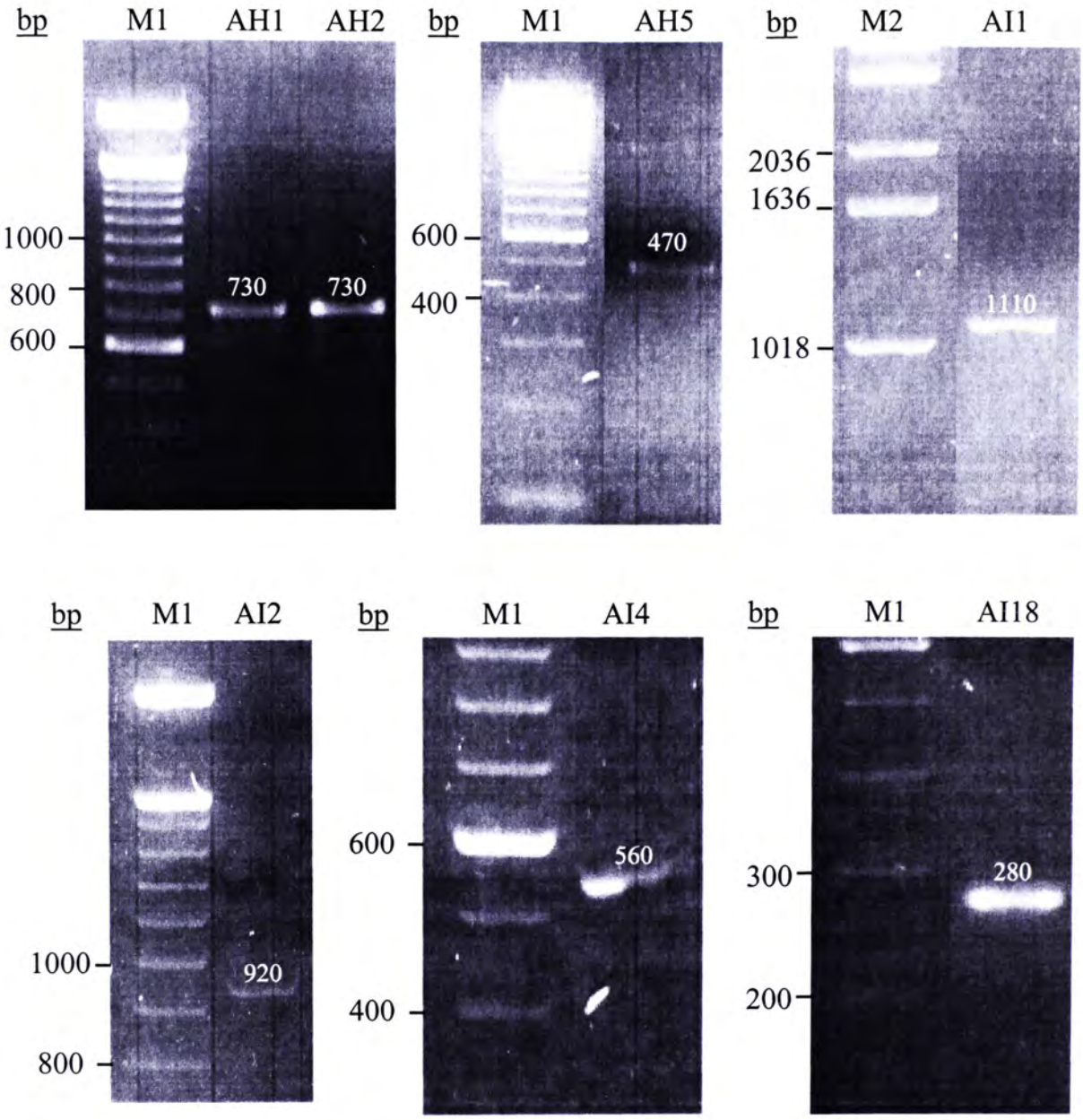

Figure 3.8.6. Reamplification of cDNA fragments excised from fluoroDD gels AH (AP11 and ARP19) and AI (AP6 and ARP4). The cDNA fragments excised from the gel were amplified by PCR using full-length M13 reverse (-48) 24-mer (5'-AGCGGATAACAATTTCACACAGGA-3') and T7 promoter 22-mer (5'-GTAATACGACTCACTATAGGGC-3') primers. The PCR products were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. M1, 100 bp DNA marker; M2, 1 kb DNA marker.

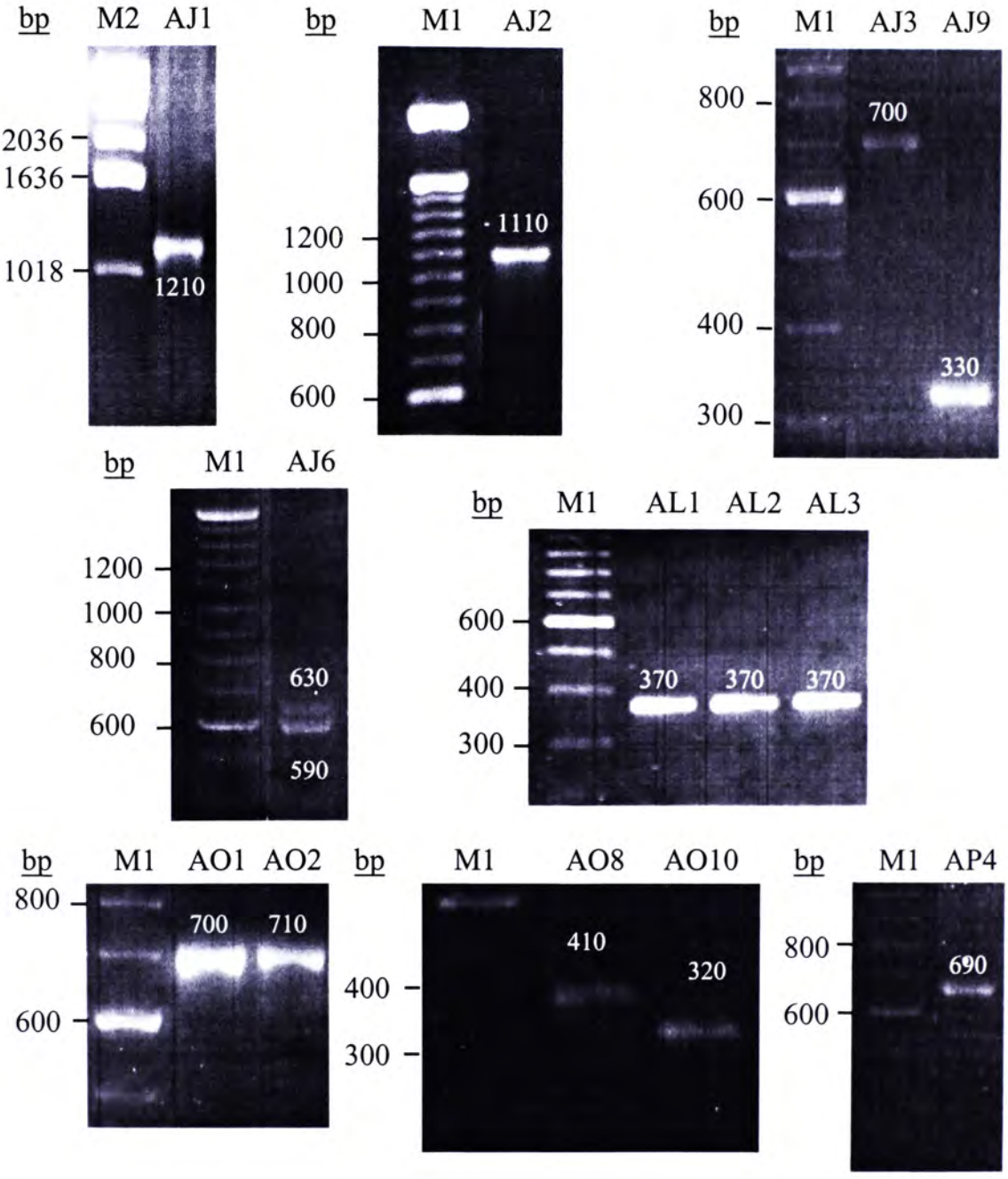

Figure 3.8.7. Reamplification of cDNA fragments excised from fluoroDD gels AJ (AP6 and ARP14), AL (AP7 and ARP15), AO (AP5 and ARP10) and AP (AP12 and ARP2). The cDNA fragments excised from the gel were amplified by PCR using full-length M13 reverse (-48) 24-mer (5'-AGCGGATAACAATTTCACACAGGA-3') and T7 promoter 22-mer (5'-GTAATACGACTCACTATAGGGC-3') primers. The PCR products were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. M1, 100 bp DNA marker; M2, 1 kb DNA marker.

Table 3.8.4. Summary of reamplified cDNA fragments excised from gels AH (AP11 and ARP19), AI (AP6 and ARP4) and AJ (AP6 and ARP14)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Differential display pattern				Reamplified fragment size (bp)
			PPARα (+/+)		PPARα (-/-)		
			CTL	Wy	CTL	Wy	
AH (AP11 & ARP19)	AH1	720	+	-	+	+	730
	AH2	700	+	-	+	+	730
	AH5	450	+	++	+	+	470
AI (AP6 & ARP4)	AI1	1100	+	-	+	+	1110
	AI2	910	+	++	+	+	920
	AI4	540	++	+	++	++	560
	AI18	270	++	+	++	++	280
AJ (AP6 & ARP14)	AJ1	1200	++	+	++	++	1210
	AJ2	1100	+	++	+	+	1110
	AJ3	680	+	++	+	+	700
	AJ6	570	++	+	++	++	590+630
	AJ9	320	+	-	+	+	330

Table 3.8.5. Summary of reamplified cDNA fragments excised from gels AL (AP7 and ARP15), AO (AP5 and ARP10) and AP (AP12 and ARP2)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Differential display pattern				Reamplified fragment size (bp)
			PPARα (+/+)		PPARα (-/-)		
			CTL	Wy	CTL	Wy	
AL (AP7 & ARP15)	AL1	355	+	++	+	+	370
	AL2	350	+	++	+	+	370
	AL3	345	+	++	+	+	370
AO (AP5 & ARP10)	AO1	700	+	++	+	+	700
	AO2	695	+	++	+	+	710
	AO8	400	+	-	+	+	410
	AO10	310	+	++	+	+	320
AP (AP12 & ARP2)	AP4	680	+	++	+	+	690

3.9 Subcloning of reamplified fluoroDD fragments

A total of forty-two reamplifed cDNA fragments were chosen for subcloning. For the cDNA fragments which subcloned into pT-Adv vector, the sizes of the inserts released by *Eco*RI digestion were 18 bp longer than the reamplifed PCR products as extra 8 nucleotides were added before and after T/A cloning site and 10 nucleotides were added after *Eco*RI digestion. They included AA1, AA5, AA6, AA7, AA10 and AA12 (Figure 3.9.1) (Table 3.9.1); AB18 and AB26 (Figure 3.9.2) (Table 3.9.2); AC3 and AC7 (Figure3.9.4) (Table 3.9.3); and AD8 and AD9 (Figure 3.9.5) (Table 3.9.3). For the cDNA fragments that subcloned into PCR®II-TOPO®, including AA4 (Figure 3.9.1) (Table 3.9.1); AB7, AB17, AB19, AB22, AB24, AB27 and AB29 (Figures 3.9.2-3.9.3) (Table 3.9.2); AC1, AC2 and AC4 (Figure 3.9.4) (Table 3.9.3); AD6 (Figure 3.9.5) (Table 3.9.3); AF1, AF21, AF25 and AF30 (Figure 3.9.5) (Table 3.9.4); AH1, AH2, AI1 and AI18 (Figure 3.9.6) (Table 3.9.4); AJ1, AJ2 and AJ9 (Figure 3.9.7) (Table 3.9.4); AL2, AL3, AO1, AO2, AO8 and AP4 (Figure3.9.8) (Table 3.9.5), the sizes of the inserts released by *Eco*RI digestion were 20 bp longer than the reamplified PCR products as extra 10 nucleotides were added before and after T/A cloning sites, and 10 nucleotides were added after *Eco*RI digestion.

Some of the subcloned cDNA fragments showed did not release the expected insert sizes after *Eco*RI digestion. cDNA fragments AA1 (Figure 3.9.1) (Table 3.9.1); AB17 and AB19 (Figure 3.9.2) (Table 3.9.2); AC1 and AC2 (Figure 3.9.4) (Table 3.9.3); AD6 (Figure 3.9.5) (Table 3.9.3) and AO2 (Figure 3.9.8) (Table 3.9.5) contained different subclones that showed different *Eco*RI restriction patterns while cDNA fragments AA1, AA6 and AA7 (Figure 3.9.1) (Table 3.9.1); AC2 (Figure 3.9.4) (Table 3.9.3); AD8 (Figure 3.9.5) (Table 3.9.3); and AO2 (Figure 3.9.8) (Table 3.9.5) showed larger insert sizes after *Eco*RI digestion. The different *Eco*RI restriction

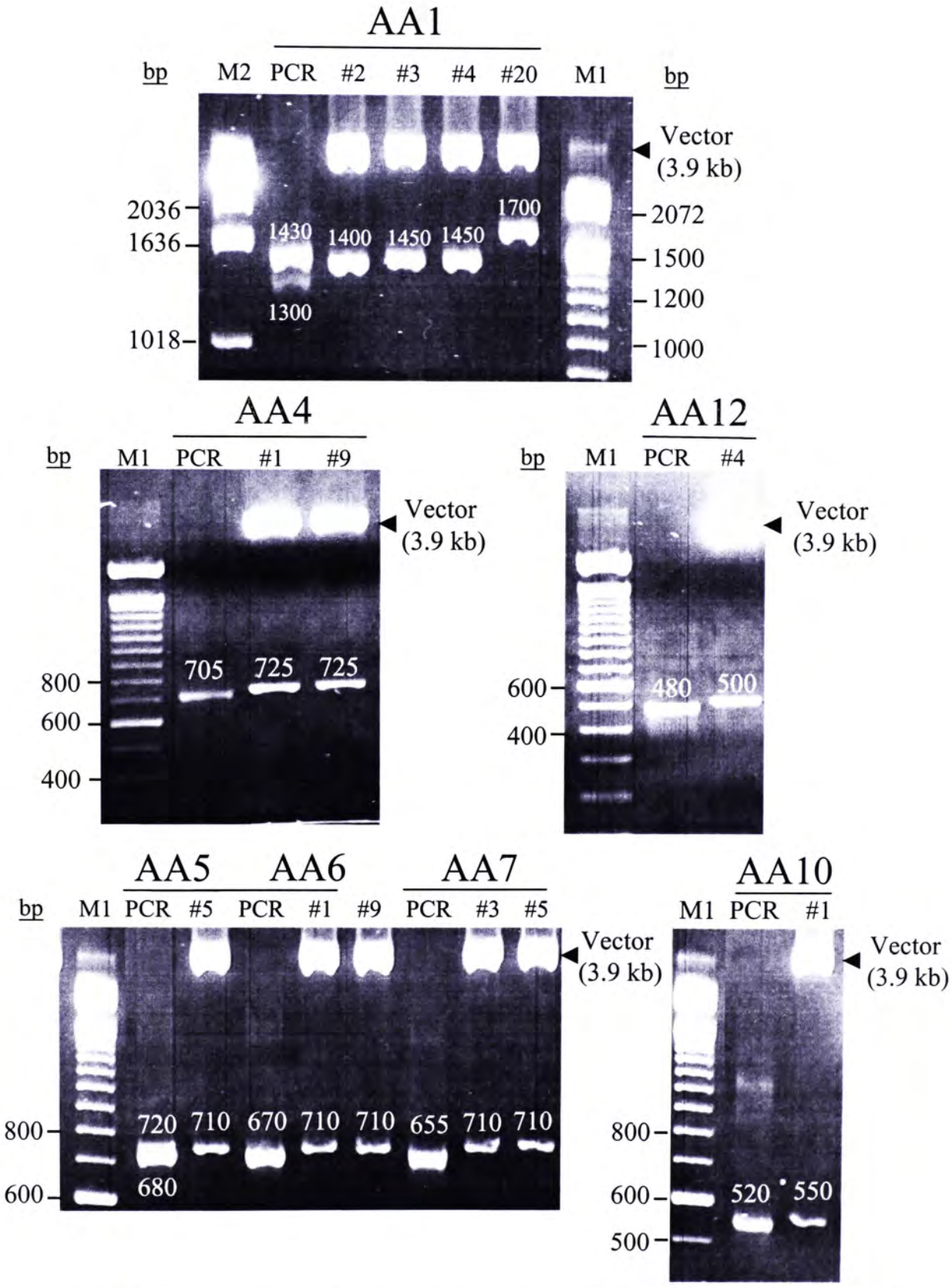

Figure 3.9.1. *Eco*RI digested cDNA fragments excised from gel AA (AP1 and ARP2). The *Eco*RI digested cDNA fragments were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. M1, 100 bp DNA marker; M2, 1 kb DNA marker. PCR, reamplified PCR product of the corresponding cDNA fragment.

Table 3.9.1. Summary of cDNA fragments subcloned from gel AA (AP1 and ARP2)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Size of vector (kb)	Size of insert after *Eco*RI cut (bp)
AA (AP1 & ARP2)	AA1	1400	1430 +1300	AA1#2	3.9 (pT-Adv)	1400
				AA1#3	3.9 (pT-Adv)	1450
				AA1#4	3.9 (pT-Adv)	1450
				AA1#20	3.9 (pT-Adv)	1700
	AA4	695	705	AA4#1	4.0 (pCR®II-TOPO®)	725
				AA4#9	4.0 (pCR®II-TOPO®)	725
	AA5	670	720+680	AA5#5	3.9 (pT-Adv)	710
	AA6	660	670	AA6#1	3.9 (pT-Adv)	710
				AA6#9	3.9 (pT-Adv)	710
	AA7	640	655	AA7#3	3.9 (pT-Adv)	710
				AA7#5	3.9 (pT-Adv)	710
	AA10	500	520	AA10#1	3.9 (pT-Adv)	550
	AA12	470	480	AA12#4	3.9 (pT-Adv)	500

Phenol:chloroform extraction was performed for screening the subclones which contained inserts. Those subclones containing plasmid DNA larger then the vector only (3.9 or 4.0 kb) were selected for performing *Eco*RI cut to confirm correct insert sizes and further used for sequencing and Northern blot analysis.

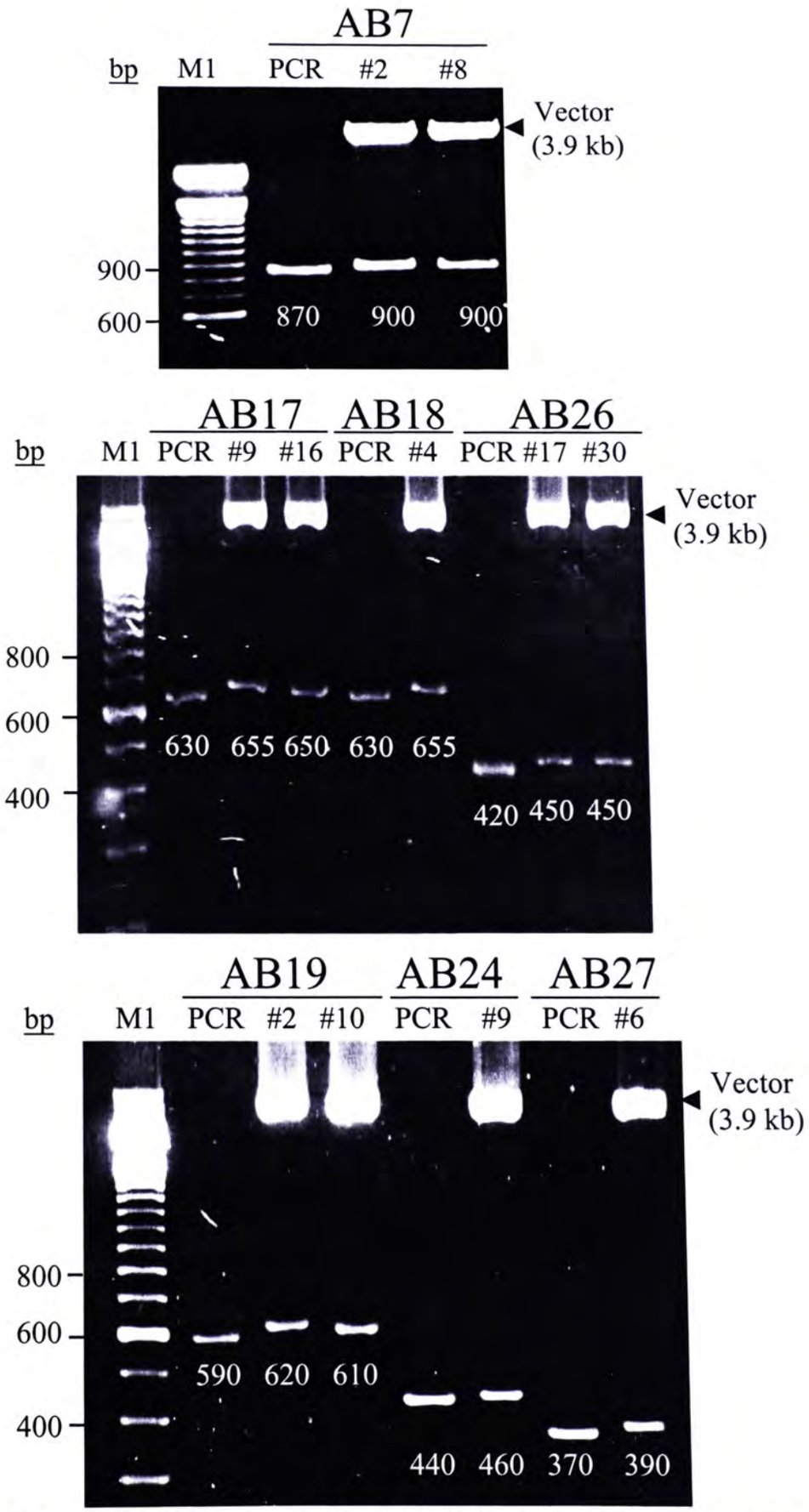

Figure 3.9.2. *Eco*RI digested cDNA fragments excised from gel AB (AP3 and ARP3) Part I. The *Eco*RI digested cDNA fragments were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. M1, 100 bp DNA marker. PCR, reamplified PCR product of the corresponding cDNA fragment.

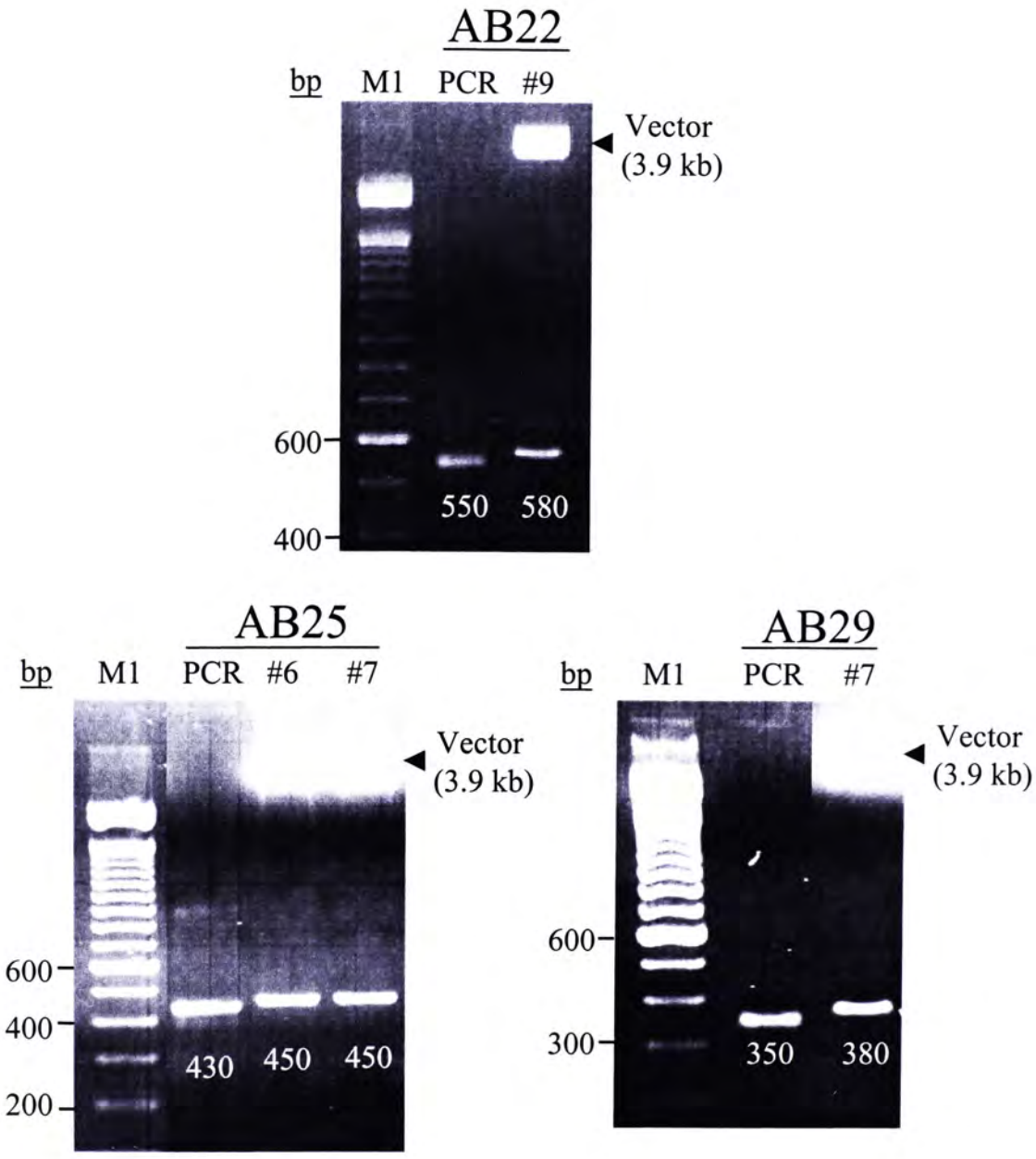

Figure 3.9.3. *Eco*RI digested cDNA fragments excised from gel AB (AP3 and ARP3) Part II. The *Eco*RI digested cDNA fragments were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. M1, 100 bp DNA marker. PCR, reamplified PCR product of the corresponding cDNA fragment.

Table 3.9.2. Summary of cDNA fragments subcloned from gel AB (AP3 and ARP3)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Size of vector (kb)	Size of insert after *Eco*RI cut (bp)
AB (AP3 & ARP3)	AB7	850	870	AB7#2	4.0 (pCR®II-TOPO®)	900
				AB7#8	4.0 (pCR®II-TOPO®)	900
	AB17	620	630	AB17#9	4.0 (pCR®II-TOPO®)	655
				AB17#16	4.0 (pCR®II-TOPO®)	650
	AB18	595	630	AB18#4	3.9 (pT-Adv)	655
	AB19	575	590	AB19#2	4.0 (pCR®II-TOPO®)	620
				AB19#10	4.0 (pCR®II-TOPO®)	610
	AB22	530	550	AB22#9	4.0 (pCR®II-TOPO®)	580
	AB24	420	440	AB24#9	4.0 (pCR®II-TOPO®)	460
	AB25	405	430	AB25#6	4.0 (pCR®II-TOPO®)	450
				AB25#7	4.0 (pCR®II-TOPO®)	450
	AB26	390	420	AB26#17	3.9 (pT-Adv)	450
				AB26#30	3.9 (pT-Adv)	450
	AB27	355	370	AB27#6	4.0 (pCR®II-TOPO®)	390
	AB29	335	350	AB29#7	4.0 (pCR®II-TOPO®)	380

Phenol:chloroform extraction was performed for screening the subclones which contained inserts. Those subclones containing plasmid DNA larger then the vector only (3.9 or 4.0 kb) were selected for performing *Eco*RI cut to confirm correct insert sizes and further used for sequencing and Northern blot analysis.

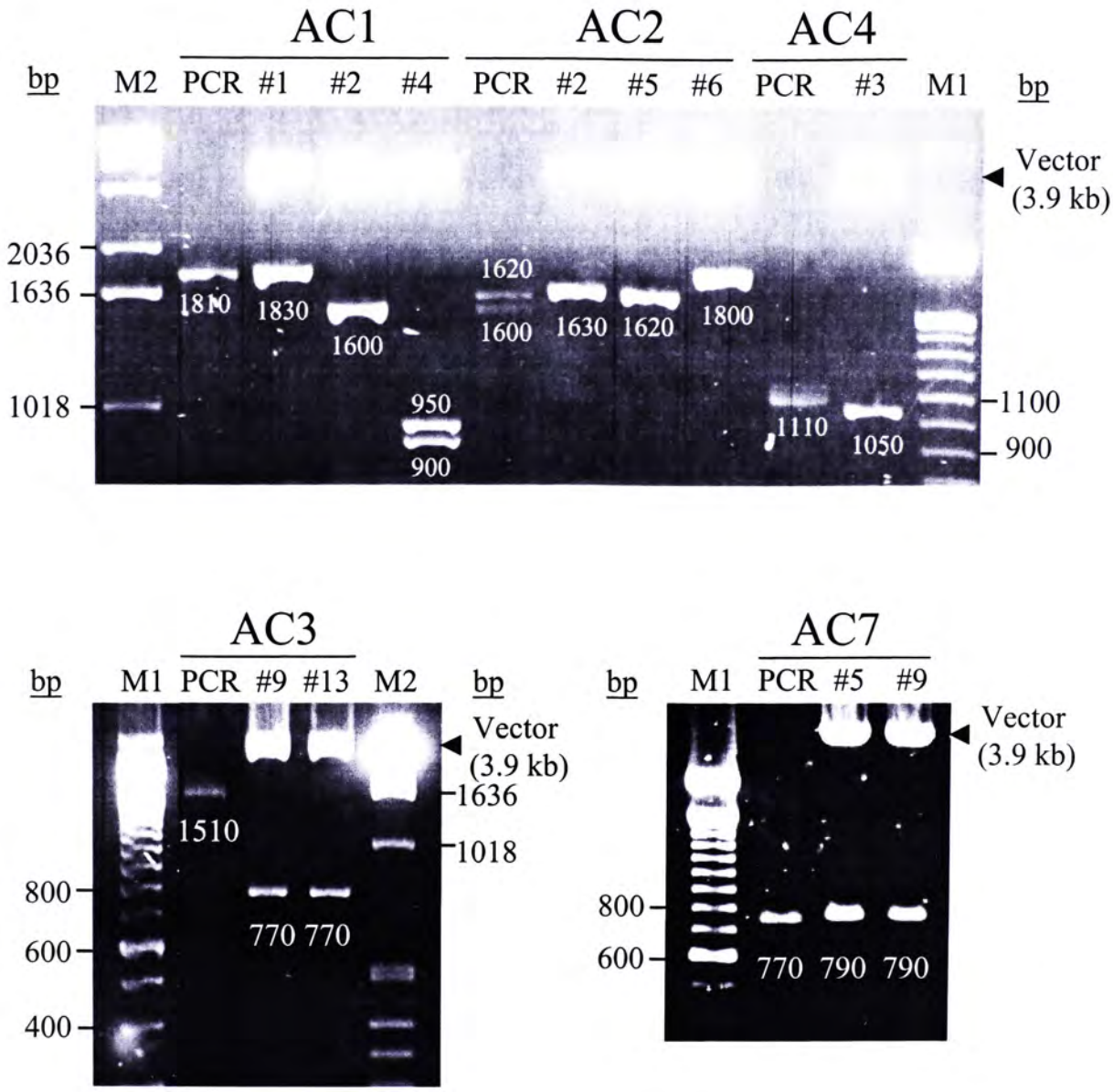

Figure 3.9.4. *Eco*RI digested cDNA fragments excised from gel AC (AP2 and ARP19). The *Eco*RI digested cDNA fragments were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. M1, 100 bp DNA marker; M2, 1 kb DNA marker. PCR, reamplified PCR product of the corresponding cDNA fragment.

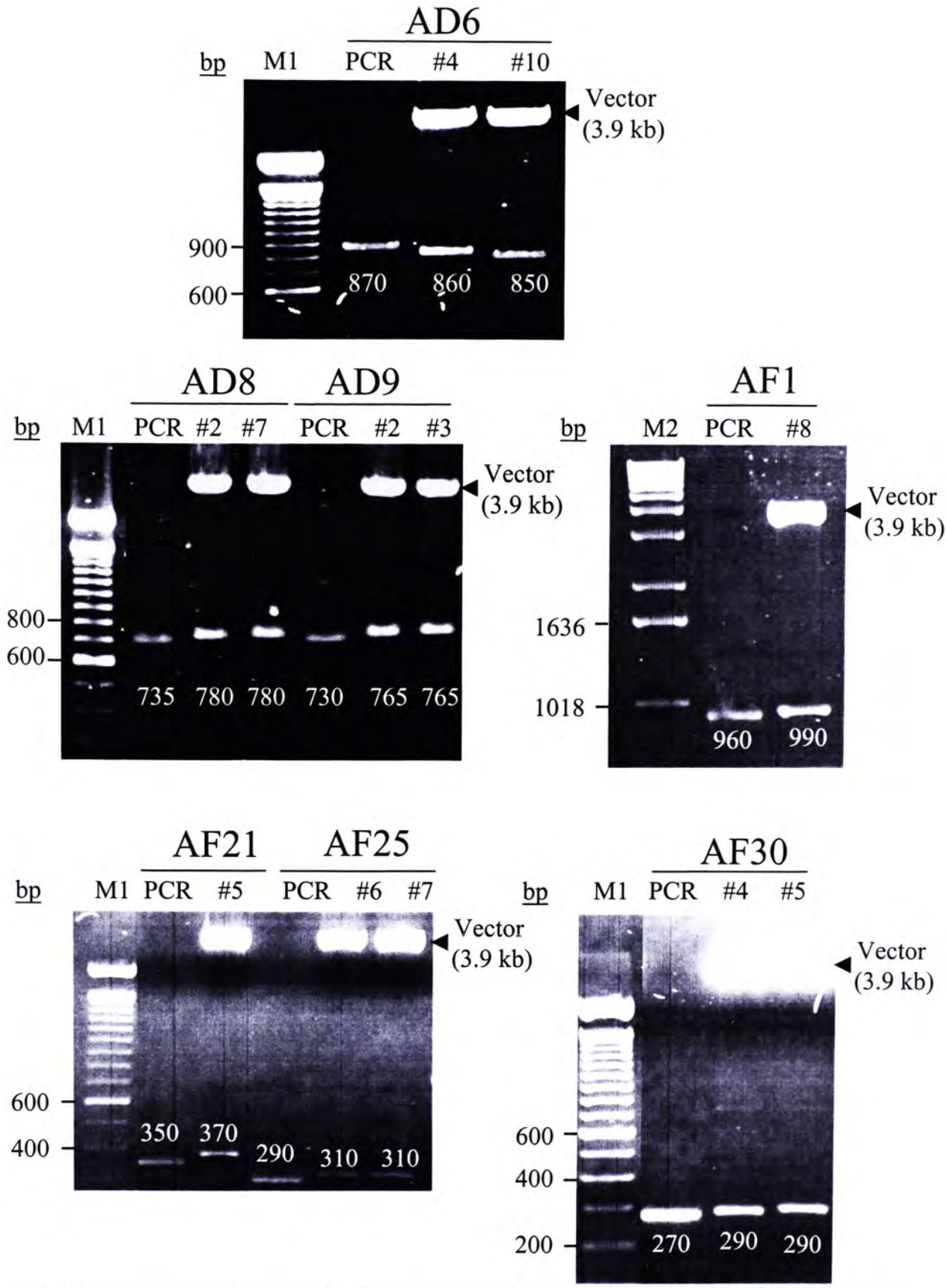

Figure 3.9.5. *Eco*RI digested cDNA fragments excised from gels AD (AP2 and ARP18) and AF (AP10 and ARP13). The *Eco*RI digested cDNA fragments were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. M1, 100 bp DNA marker; M2, 1 kb DNA marker. PCR, reamplified PCR product of the corresponding cDNA fragment.

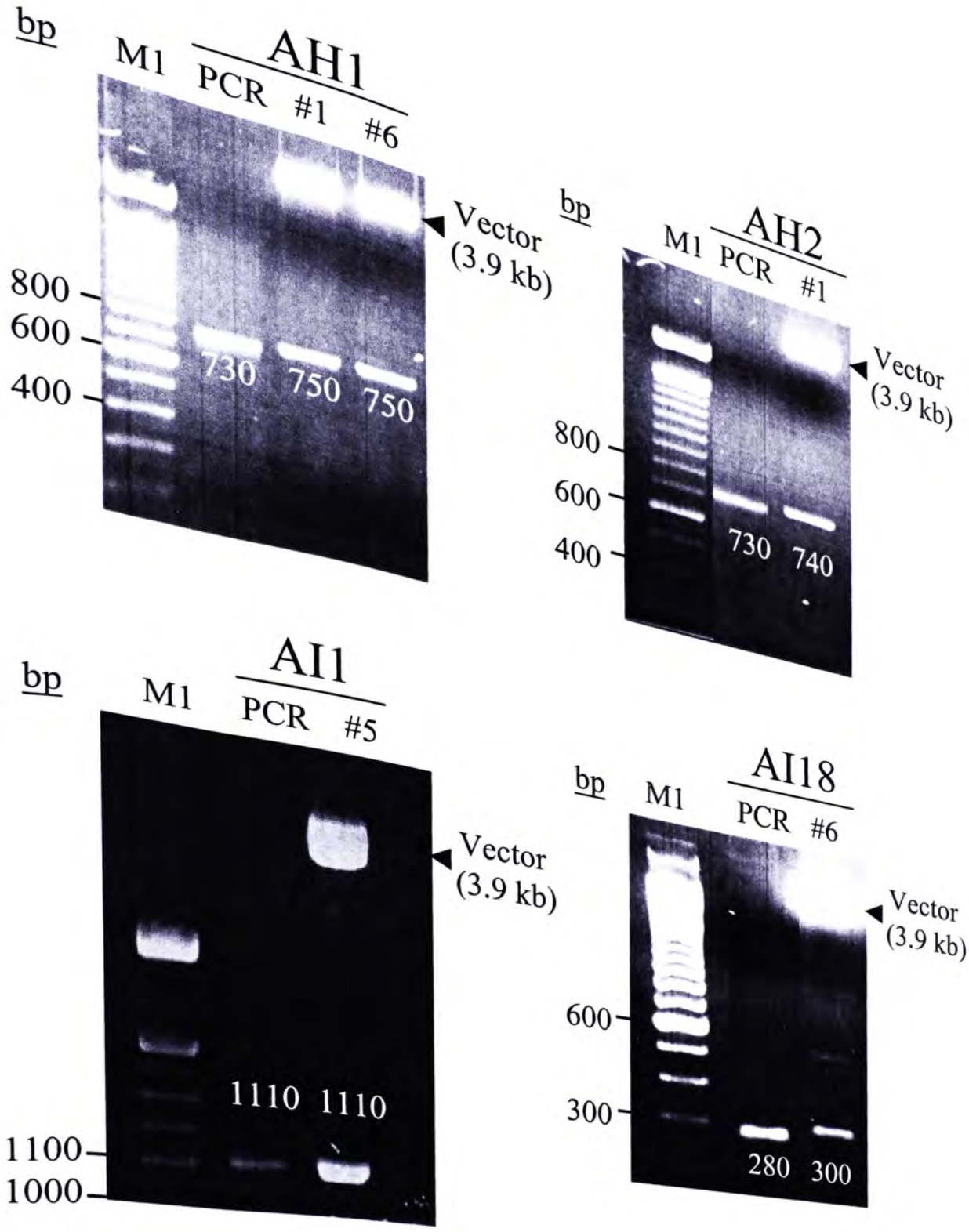

Figure 3.9.6. *Eco*RI digested cDNA fragments excised from gels AH (AP11 and ARP19) and AI (AP6 and ARP4). The *Eco*RI digested cDNA fragments were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. M1, 100 bp DNA marker. PCR, reamplified PCR product of the corresponding cDNA fragment.

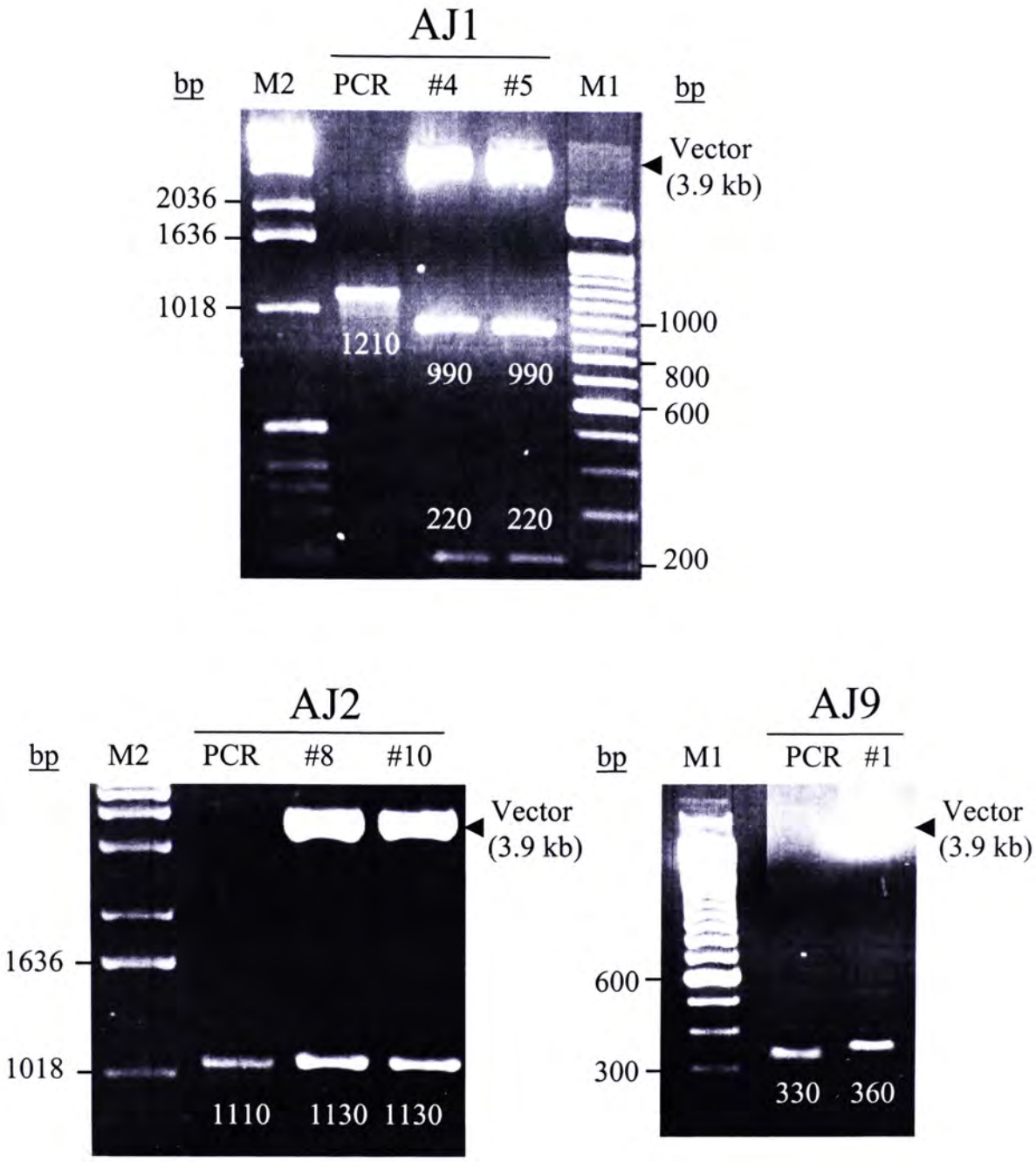

Figure 3.9.7. *Eco*RI digested cDNA fragments excised from gel AJ (AP6 and ARP14). The *Eco*RI digested cDNA fragments were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. M1, 100 bp DNA marker; M2, 1 kb DNA marker. PCR, reamplified PCR product of the corresponding cDNA fragment.

Table 3.9.3. Summary of cDNA fragments subcloned from gels AC (AP2 and ARP19) and AD (AP2 and ARP18)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Size of vector (kb)	Size of insert after *Eco*RI cut (bp)
AC (AP2 & ARP19)	AC1	1800	1810	AC1#1	4.0 (pCR®II-TOPO®)	1830
				AC1#2	4.0 (pCR®II-TOPO®)	1600
				AC1#4	4.0 (pCR®II-TOPO®)	900+950
	AC2	1600	1620 +1600	AC2#2	4.0 (pCR®II-TOPO®)	1630
				AC2#5	4.0 (pCR®II-TOPO®)	1620
				AC2#6	4.0 (pCR®II-TOPO®)	1800
	AC3	1500	1510	AC3#9	3.9 (pT-Adv)	770+770
				AC3#13	3.9 (pT-Adv)	770+770
	AC4	1100	1110	AC4#3	4.0 (pCR®II-TOPO®)	1050
	AC7	770	770	AC7#5	3.9 (pT-Adv)	790
				AC7#9	3.9 (pT-Adv)	790
AD (AP2 & ARP18)	AD6	840	870	AD6#4	4.0 (pCR®II-TOPO®)	860
				AD6#10	4.0 (pCR®II-TOPO®)	850
	AD8	720	735	AD8#2	3.9 (pT-Adv)	780
				AD8#7	3.9 (pT-Adv)	780
	AD9	710	730	AD9#2	3.9 (pT-Adv)	765
				AD9#3	3.9 (pT-Adv)	765

Phenol:chloroform extraction was performed for screening the subclones which contained inserts. Those subclones containing plasmid DNA larger then the vector only (3.9 or 4.0 kb) were selected for performing *Eco*RI cut to confirm correct insert sizes and further used for sequencing and Northern blot analysis.

Table 3.9.4. Summary of cDNA fragments subcloned from gels AF (AP10 and ARP13), AH (AP11 and ARP19), AI (AP6 and ARP4) and AJ (AP6 and ARP14)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Size of vector (kb)	Size of insert after *Eco*RI cut (bp)
AF (AP10 & ARP13)	AF1	945	960	AF1#8	4.0 (pCR®II-TOPO®)	990
	AF21	340	350	AF21#5	4.0 (pCR®II-TOPO®)	370
	AF25	275	290	AF25#6	4.0 (pCR®II-TOPO®)	310
				AF25#7	4.0 (pCR®II-TOPO®)	310
	AF30	240	270	AF30#4	4.0 (pCR®II-TOPO®)	290
				AF30#5	4.0 (pCR®II-TOPO®)	290
AH (AP11 & ARP19)	AH1	720	730	AH1#1	4.0 (pCR®II-TOPO®)	750
				AH1#6	4.0 (pCR®II-TOPO®)	750
	AH2	700	730	AH2#1	4.0 (pCR®II-TOPO®)	740
AI (AP6 & ARP4)	AI1	1100	1110	AI1#5	4.0 (pCR®II-TOPO®)	1110
	AI18	270	280	AI18#6	4.0 (pCR®II-TOPO®)	300
AJ (AP6 & ARP14)	AJ1	1200	1210	AJ1#4	4.0 (pCR®II-TOPO®)	990+220
				AJ1#5	4.0 (pCR®II-TOPO®)	990+220
	AJ2	1100	1110	AJ2#8	4.0 (pCR®II-TOPO®)	1130
				AJ2#10	4.0 (pCR®II-TOPO®)	1130
	AJ9	320	330	AJ9#1	4.0 (pCR®II-TOPO®)	360

Phenol:chloroform extraction was performed for screening the subclones which contained inserts. Those subclones containing plasmid DNA larger then the vector only (3.9 or 4.0 kb) were selected for performing *Eco*RI cut to confirm correct insert sizes and further used for sequencing and Northern blot analysis.

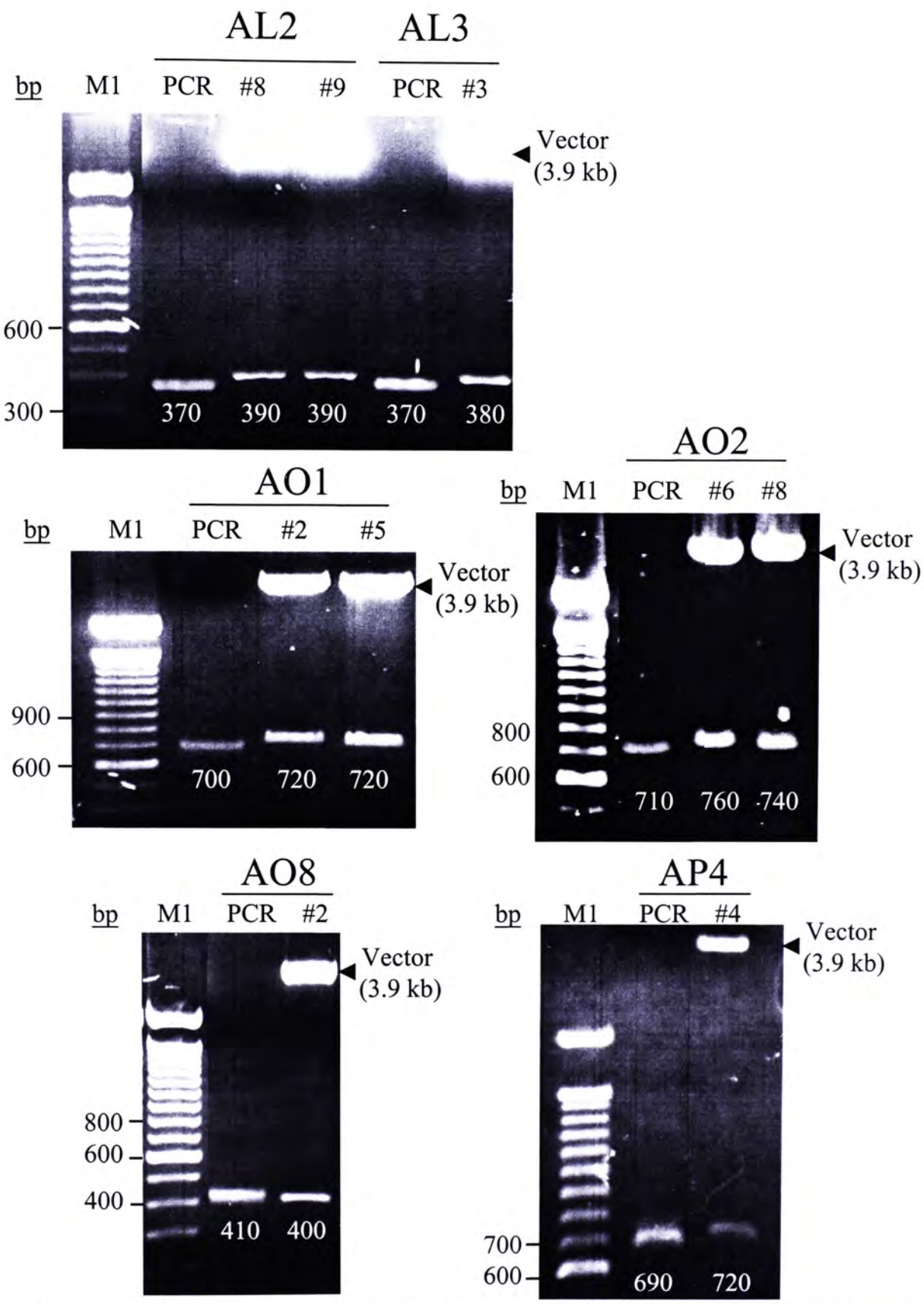

Figure 3.9.8. *Eco*RI digested cDNA fragments excised from gels AL (AP7 and ARP15), AO (AP5 and ARP10) and AP (AP12 and ARP2). The *Eco*RI digested cDNA fragments were resolved on 1% agarose, 0.5X TBE gels with ethidium bromide staining. M1, 100 bp DNA marker. PCR, reamplified PCR product of the corresponding cDNA fragment.

Table 3.9.5. Summary of cDNA fragments subcloned from gels AL (AP7 and ARP15), AO (AP5 and ARP10) and AP (AP12 and ARP2)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Size of vector (kb)	Size of insert after *Eco*RI cut (bp)
AL (AP7 & ARP15)	AL2	350	370	AL2#8	4.0 (pCR®II-TOPO®)	390
				AL2#9	4.0 (pCR®II-TOPO®)	390
	AL3	345	370	AL3#3	4.0 (pCR®II-TOPO®)	380
AO (AP5 & ARP10)	AO1	700	700	AO1#2	4.0 (pCR®II-TOPO®)	720
				AO1#5	4.0 (pCR®II-TOPO®)	720
	AO2	695	710	AO2#6	4.0 (pCR®II-TOPO®)	760
				AO2#8	4.0 (pCR®II-TOPO®)	740
	AO8	400	410	AO8#2	4.0 (pCR®II-TOPO®)	400
AP (AP12 & ARP2)	AP4	680	690	AP4#4	4.0 (pCR®II-TOPO®)	720

Phenol:chloroform extraction was performed for screening the subclones which contained inserts. Those subclones containing plasmid DNA larger then the vector only (3.9 or 4.0 kb) were selected for performing *Eco*RI cut to confirm correct insert sizes and further used for sequencing and Northern blot analysis.

patterns and larger insert sizes might be due to contamination and/or nearby co-migrated cDNA fragments. In contrast, cDNA fragments AA1 (Figure 3.9.1) (Table 3.9.1); AC1, AC3 and AC4 (Figure 3.9.4) (Table 3.9.3); AD6 (Figure 3.9.5) (Table 3.9.3); AI1 (Figure 3.9.6) (Table 3.9.4); AJ1 (Figure 3.9.7) (Table3.9.4) and AO8 (Figure 3.9.8) (Table 3.9.5) showed smaller insert sizes and these might be due to the presence of *Eco*RI site in those inserts. In addition, contamination of nearby co-migrated cDNA fragments or other cDNA fragments during bands excision would also give small sizes of inserts after *Eco*RI digestion.

3.10 Sequencing of subcloned cDNA fragments

A total of forty-two subcloned cDNA fragments (seventy subclones) were sequenced and twenty-six different know genes and one expressed sequence tags (EST) were obtained. They included mouse peroxisomal delta3, delta2-enoyl-Coenzyme A isomerase (Peci) [subclones AA1#2, AA1#3, AA1#4 and #20 (Table 3.10.1)]; mouse apolipoprotein A-V (Apoa5) [subclones AA4#1 and AA4#9 (Table 3.10.1)]; mouse complete mitochondrial genome (mitochondria) [subclones AA5#5, AA6#1 and AA6#9 (Table3.10.1); AA7#3 and AA7#5 (Table 3.10.2); AB17#16 and AB18#4 (Table 3.10.3); AB19#2 and AB19#10 (Table 3.10.4); AF30#5 (Table 3.10.11) and AP4#4 (Table 3.10.16)]; mouse cysteine sulfinic acid decarboxylase (Csad) [subclone AA10#1 (Table 3.10.2)]; mouse acetyl-Coenzyme A dehydrogenase, medium chain (MCAD) [subclone AA12#4 (Table 3.10.2)]; mouse UDP-glucuronosyltransferase (UGT2b5) [subclones AB7#2 and AB7#8 (Table 3.10.3)]; mouse peroxisome biogenesis factor 16 (Pex 16) [subclone AB22#9 (Table 3.10.4)]; mouse cytochrome P450 (Cyp4a14) [subclones AB24#9 and AB25#6 (Table 3.10.4); AB26#17 and AB26#30 (Table 3.10.5)], mouse catalase [subclones AB29#7 (Table 3.10.5); AC2#5

(Table 3.10.7) and AJ9#1 (Table 3.10.13)]; mouse serine (or cysteine) proteinase inhibitor, clade F, member 2 (Serpinf2) [subclones AC1#1 (Table 3.10.6) and AC2#6 (Table 3.10.7)]; serine (or cysteine) proteinase inhibitor, clade A, member 1a (SPI) [subclone AC1#2 (Table 3.10.6); AC4#3 and AC7#5 (Table 3.10.8)]; serine (or cysteine) proteinase inhibitor, clade A, member 3K (Spi2) [subclone AI1#5 (Table 3.10.12)]; mouse peroxisomal bifunctional enzyme (PBFE) [subclone AC2#2 (Table 3.10.7)]; mouse cytochrome P450 2a5 (Cyp2a5) [subclone AC4#3 (Table 3.10.8)]; mouse N-terminal Asn amidase [subclone AD6#4 (Table 3.10.9)]; mouse cytochrome P450 4a10 (Cyp4a10) [subclones AD6#10, AD8#2, AD8#7, AD9#2 and AD9#3 (Table 3.10.9)]; mouse very-long-chain acyl-CoA synthetase (VLACS) [subclone AF1#8 (Table 3.10.10)]; mouse cell death-inducing DNA fragmentation factor, alpha subunit-like effector B (Cidef) [subclone AF21#5 (Table 3.10.10)]; mouse major urinary protein II (MUP II) [subclone AF25#6 and AF25#7 (Table 3.10.10)]; mouse suppressor of actin mutations (SAC1 gene) [subclone AF30#4 (Table 3.10.11) and AJ9#1 (Table 3.10.13)]; mouse expressed sequence tags (EST) [subclone AH1#1 (Table 3.10.11)]; mouse argininosuccinate lyase (Asl) [subclone AI18#6 (Table 3.10.12)]; mouse carboxyesterase [subclones AJ1#4 and AJ1#5 (Table 3.10.13)]; mouse peroxisomal acyl-CoA oxidase (AOX) [subclone AJ2#10 (Table 3.10.13)]; mouse hydroxysteroid (17-beta) dehydrogenase 11 (Hsd17β11) [subclones AL2#8 and AL3#3 (Table 3.10.14)]; mouse adipose differentiation related protein (ADRP) [subclone AO1#2 (Table 3.10.15)]; mouse carnitine O-octanoyltransferase (Crot) [subclones AO1#5 and AO2#8 (Table 3.10.15)]; mouse ribonuclease, RNase A family 4 (RNase4) [subclones AO2#6 and AO8#2 (Table 3.10.15)] (Appendix B, B1.1-16.1).

The abundance of genes were shown in Figure 3.10.1, including mitochondrial (19.1%) > SPI (7.1%), Catalase (7.1%), Cyp4a10 (7.1%) and Cyp4a14 (7.1%) > Crot

Table 3.10.1. DNA sequences of cDNA fragments subcloned from gel AA (AP1 and ARP2) part I

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Sequencing				
					Size of insert (bp)	Sequencing primer	Gene homology (Transcript size)	Identities	E value
AA (AP1 & ARP2)	AA1	1400	1430 +1300	AA1#2*	1400	M13 forward (-20)	*Mus musculus* peroxisomal delta 3, delta 2-enoyl-coenzyme A isomerase, Peci (1581 bp)	447/474 (94%)	0
				AA1#3	1450	M13 forward (-20)		187/191 (97%)	3e-85
				AA1#4	1450	M13 reverse		199/209 (95%)	8e-80
				AA1#20	1700	M13 forward (-20)		541/569 (95%)	0
	AA4	695	705	AA4#1	725	M13 forward (-20)	*Mus musculus* apolipoprotein A-V, Apoa5 (1819 bp)	112/121 (92%)	1e-32
				AA4#9*	725	M13 reverse		231/245 (94%)	3e-95
	AA5	670	720+680	AA5#5	710	M13 forward (-20)	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	496/524 (94%)	0
	AA6	660	670	AA6#1	710	M13 forward (-20)	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	495/517 (95%)	0
				AA6#9	710	M13 reverse		563/579 (97%)	0

M13 (-20) forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers were used for sequencing. After sequencing, sequence homology of the identified genes was searched against the known genes and ESTs in GenBank using BLAST. The identities indicate the percentage of the cDNA fragment match with the BLAST search sequence, while the expect value (E) is the parameter to describe the probability of the cDNA fragment that occurred in the database by chance. *Subclones selected for subsequent Northern blot analysis.

Table 3.10.2. DNA sequences of cDNA fragments subcloned from gel AA (AP1 and ARP2) part II

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Sequencing				
					Size of insert (bp)	Sequencing primer	Gene homology (Transcript size)	Identities	E value
AA (AP1 & ARP2)	AA7	640	655	AA7#3	710	M13 forward (-20)	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	495/522 (94%)	0
				AA7#5	710	M13 reverse		440/454 (96%)	0
	AA10	500	520	AA10#1*	550	M13 forward (-20)	*Mus musculus* cysteine sulfinic acid decarboxylase, Csad (2265 bp)	304/311 (97%)	e-143
						M13 reverse		163/182 (89%)	3e-32
	AA12	470	480	AA12#4*	500	M13 forward (-20)	*Mus musculus* acetyl-coenzyme A dehydrogenase, medium chain, MCAD (1857 bp)	419/419 (100%)	0
						M13 reverse		100/103 (97%)	5e-38

M13 (-20) forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers were used for sequencing. After sequencing, sequence homology of the identified genes was searched against the known genes and ESTs in GenBank using BLAST. The identities indicate the percentage of the cDNA fragment match with the BLAST search sequence, while the expect value (E) is the parameter to describe the probability of the cDNA fragment that occurred in the database by chance. *Subclones selected for subsequent Northern blot analysis.

Table 3.10.3. DNA sequences of cDNA fragments subcloned from gel AB (AP3 and ARP3) part I

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Sequencing				
					Size of insert (bp)	Sequencing primer	Gene homology (Transcript size)	Identities	E value
AB (AP3 & ARP3)	AB7	850	870	AB7#2*	900	M13 forward (-20)	Mouse UDP-glucuronosyltransferase 2 family, member 5, UGT2b5 (1648 bp)	584/642 (90%)	0
				AB7#8	900	M13 reverse		482/534 (90%)	0
	AB17	620	630	AB17#9	655	M13 forward (-20)	Failed in sequencing		
				AB17#16	650	M13 reverse	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	478/496 (96%)	0
	AB18	595	630	AB18#4	655	M13 forward (-20)	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	477/490 (97%)	0
						M13 reverse		524/538 (97%)	0

M13 (-20) forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers were used for sequencing. After sequencing, sequence homology of the identified genes was searched against the known genes and ESTs in GenBank using BLAST. The identities indicate the percentage of the cDNA fragment match with the BLAST search sequence, while the expect value (E) is the parameter to describe the probability of the cDNA fragment that occurred in the database by chance. *Subclones selected for subsequent Northern blot analysis.

Table 3.10.4. DNA sequences of cDNA fragments subcloned from gel AB (AP3 and ARP3) part II

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Sequencing				
					Size of insert (bp)	Sequencing primer	Gene homology (Transcript size)	Identities	E value
AB (AP3 & ARP3)	AB19	575	590	AB19#2	620	M13 forward (-20)	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	523/530 (98%)	0
				AB19#10	610	M13 reverse		513/516 (99%)	0
	AB22	530	550	AB22#9*	580	M13 forward (-20)	*Mus musculus* peroxisome biogenesis factor 16, Pex16 (1221 bp)	405/430 (94%)	e-178
						M13 reverse		482/486 (99%)	0
	AB24	420	440	AB24#9	460	M13 forward (-20)	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 14, Cyp4a14 (2547 bp)	354/355 (99%)	0
						M13 reverse		331/339 (97%)	e-165
	AB25	405	430	AB25#6*	450	M13 forward (-20)	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 14, Cyp4a14 (2547 bp)	150/162 (92%)	7e-47
				AB25#7	450	M13 reverse	Failed in sequencing		

M13 (-20) forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers were used for sequencing. After sequencing, sequence homology of the identified genes was searched against the known genes and ESTs in GenBank using BLAST. The identities indicate the percentage of the cDNA fragment match with the BLAST search sequence, while the expect value (E) is the parameter to describe the probability of the cDNA fragment that occurred in the database by chance. *Subclones selected for subsequent Northern blot analysis.

Table 3.10.5. DNA sequences of cDNA fragments subcloned from gel AB (AP3 and ARP3) part III

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Sequencing				
					Size of insert (bp)	Sequencing primer	Gene homology (Transcript size)	Identities	E value
AB (AP3 & ARP3)	AB26	390	420	AB26#17	450	M13 forward (-20)	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 14, Cyp4a14 (2547 bp)	354/355 (99%)	0
				AB26#30	450	M13 reverse		353/356 (99%)	0
	AB27	355	370	AB27#6	390	M13 forward (-20)	Failed in sequencing		
						M13 reverse	Failed in sequencing		
	AB29	335	350	AB29#7	380	M13 forward (-20)	if84a04.x1 Kaestner ngn3 - - subtracted *Mus musculus* cDNA 3'similar to SW:CATA_CAMJE Q59296 CATALASE (283 bp)	242/253 (95%)	e-114
						M13 reverse	Failed in sequencing		

M13 (-20) forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers were used for sequencing. After sequencing, sequence homology of the identified genes was searched against the known genes and ESTs in GenBank using BLAST. The identities indicate the percentage of the cDNA fragment match with the BLAST search sequence, while the expect value (E) is the parameter to describe the probability of the cDNA fragment that occurred in the database by chance. *Subclones selected for subsequent Northern blot analysis.

Table 3.10.6. DNA sequences of cDNA fragments subcloned from gel AC (AP2 and ARP19) part I

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Sequencing				
					Size of insert (bp)	Sequencing primer	Gene homology (Transcript size)	Identities	E value
AC (AP2 & ARP19)	AC1	1800	1810	AC1#1	1830	M13 forward (-20)	*Mus musculus* serine (or cysteine) proteinase inhibitor, clade F, member 2, Serpinf2 (2187 bp)	495/503 (98%)	0
						M13 reverse		119/123 (96%)	7e-52
				AC1#2*	1600	M13 forward (-20)	*Mus musculus* serine (or cysteine) proteinase inhibitor, clade A, member 1a, SPI (1393bp)	406/422 (96%)	0
						M13 reverse		237/241 (98%)	e-116
				AC1#4	900 + 950	M13 reverse	Failed in sequencing		

M13 (-20) forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers were used for sequencing. After sequencing, sequence homology of the identified genes was searched against the known genes and ESTs in GenBank using BLAST. The identities indicate the percentage of the cDNA fragment match with the BLAST search sequence, while the expect value (E) is the parameter to describe the probability of the cDNA fragment that occurred in the database by chance. *Subclones selected for subsequent Northern blot analysis.

Table 3.10.7. DNA sequences of cDNA fragments subcloned from gel AC (AP2 and ARP 19) part II

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Sequencing				
					Size of insert (bp)	Sequencing primer	Gene homology (Transcript size)	Identities	E value
AC (AP2 & ARP19)	AC2	1600	1620+1600	AC2#2*	1630	M13 reverse	*Mus musculus* peroxisomal bifunctional enzyme (PBE) (PBFE) [includes: enoyl-CoA dydratase (EC 4.2.1.17); 3,2-trans_enoyl-CoA isomerase (EC 5.3.3.8); 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35)], PBFE (2979 bp)	525/545 (96%)	0
				AC2#5	1620	M13 reverse	if84h05.x1 Kaestner ngn3 - - subtracted *Mus musculus* cDNA 3' similar to SW:CATA_CAMJE Q59296 CATALASE (280 bp)	93/99 (93%)	4e-19
				AC2#6	1800	M13 forward (-20)	*Mus musculus* serine (or cysteine) proteinase inhibitor, clade F, member 2, Serpinf2 (2187 bp)	633/641 (98%)	0

M13 (-20) forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers were used for sequencing. After sequencing, sequence homology of the identified genes was searched against the known genes and ESTs in GenBank using BLAST. The identities indicate the percentage of the cDNA fragment match with the BLAST search sequence, while the expect value (E) is the parameter to describe the probability of the cDNA fragment that occurred in the database by chance. *Subclones selected for subsequent Northern blot analysis.

Table 3.10.8. DNA sequences of cDNA fragments subcloned from gel AC (AP2 and ARP19) part III

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Sequencing				
					Size of insert (bp)	Sequencing primer	Gene homology (Transcript size)	Identities	E value
AC (AP2 & ARP19)	AC3	1500	1510	AC3#9	770 + 770	M13 forward (-20)	Failed in sequencing		
				AC3#13	770 + 770	M13 reverse			
	AC4	1100	1110	AC4#3	1050	M13 forward (-20)	*Mus musculus* cytochrome P450, family 2, subfamily a, polypeptide 5, Cyp2a5 (1720 bp)	302/329 (91%)	e-121
						M13 reverse	*Mus musculus* serine (or cysteine) proteinase inhibitor, clade A, member 1a, SPI (1393 bp)	219/229 (95%)	4e-85
	AC7	770	770	AC7#5	790	M13 forward (-20)	*Mus musculus* serine (or cysteine) proteinase inhibitor, clade A, member 1a, SPI (1393 bp)	394/406 (97%)	0
				AC7#9	790	M13 reverse	Failed in sequencing		

M13 (-20) forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers were used for sequencing. After sequencing, sequence homology of the identified genes was searched against the known genes and ESTs in GenBank using BLAST. The identities indicate the percentage of the cDNA fragment match with the BLAST search sequence, while the expect value (E) is the parameter to describe the probability of the cDNA fragment that occurred in the database by chance. *Subclones selected for subsequent Northern blot analysis.

Table 3.10.9. DNA sequences of cDNA fragments subcloned from gel AD (AP2 and ARP18)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Sequencing				
					Size of insert (bp)	Sequencing primer	Gene homology (Transcript size)	Identities	E value
AD (AP2 & ARP18)	AD6	840	870	AD6#4	860	M13 forward (-20)	Failed in sequencing		
						M13 reverse	*Mus musculus* N-terminal Asn amidase, Ntanl (1184 bp)	476/494 (96%)	0
				AD6#10	850	M13 forward (-20)	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 10, Cyp4a10 (2085 bp)	460/507 (90%)	e-159
						M13 reverse		437/488 (89%)	e-155
	AD8	720	735	AD8#2*	780	M13 forward (-20)	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 10, Cyp4a10 (2085 bp)	474/515 (92%)	0
				AD8#7	780	M13 reverse		353/396 (89%)	e-105
	AD9	710	730	AD9#2	765	M13 forward (-20)	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 10, Cyp4a10 (2085 bp)	472/513 (92%)	0
				AD9#3	765	M13 reverse		398/439 (90%)	e-153

M13 (-20) forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers were used for sequencing. After sequencing, sequence homology of the identified genes was searched against the known genes and ESTs in GeneBank using BLAST. The identities indicate the percentage of the cDNA fragment match with the BLAST search sequence, while the expect value (E) is the parameter to describe the probability of the cDNA fragment that occurred in the database by chance. *Subclones selected for subsequent Northern blot analysis.

Table 3.10.10. DNA sequences of cDNA fragments subcloned from gel AF (AP10 and ARP13)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Sequencing				
					Size of insert (bp)	Sequencing primer	Gene homology (Transcript size)	Identities	E value
AF (AP10 & ARP13)	AF1	945	960	AF1#8*	990	M13 forward (-20)	*Mus musculus* very-long-chain acyl-CoA synthetase, VLACS (2209 bp)	606/623 (97%)	0
						M13 reverse		119/230 (86%)	1e-56
	AF21	340	350	AF21#5	370	M13 forward (-20)	Failed in sequencing		
						M13 reverse	*Mus musculus* cell death-inducing DNA fragmentation factor, alpha subunit-like effector B, Cideb (1182 bp)	285/292 (97%)	e-140
	AF25	275	290	AF25#6	310	M13 forward (-20)	*Mus musculus* major urinary protein 2, MUP II (891 bp)	218/223 (97%)	e-103
				AF25#7*	310	M13 reverse		217/219 (99%)	e-114

M13 (-20) forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers were used for sequencing. After sequencing, sequence homology of the identified genes was searched against the known genes and ESTs in GenBank using BLAST. The identities indicate the percentage of the cDNA fragment match with the BLAST search sequence, while the expect value (E) is the parameter to describe the probability of the cDNA fragment that occurred in the database by chance. *Subclones selected for subsequent Northern blot analysis.

Table 3.10.11. DNA sequences of cDNA fragments subcloned from gels AF (AP10 and ARP13) and AH (AP11 and ARP19)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Sequencing				
					Size of insert (bp)	Sequencing primer	Gene homology (Transcript size)	Identities	E value
AF (AP10 & ARP13)	AF30	240	270	AF30#4	290	M13 forward (-20)	*Mus musculus* suppressor of actin mutations, SAC1 gene (2029 bp)	82/85 (96%)	1e-29
				AF30#5	290	M13 reverse	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	170/173 (98%)	1e-72
AH (AP11 & ARP19)	AH1	720	730	AH1#1	750	M13 forward (-20)	vm09c03.r1 Knowles Solter mouse blastocyst B1 *Mus musculus* cDNA clone IMAGE:989668 5' similar to gb:U15647_cds1 *Mus musculus* (624 bp)	377/447 (84%)	2e-82
				AH1#6	750	M13 reverse	Failed in sequencing		
	AH2	700	730	AH2#1	740	M13 forward (-20)	Failed in sequencing		
						M13 reverse	Failed in sequencing		

M13 (-20) forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers were used for sequencing. After sequencing, sequence homology of the identified genes was searched against the known genes and ESTs in GenBank using BLAST. The identities indicate the percentage of the cDNA fragment match with the BLAST search sequence, while the expect value (E) is the parameter to describe the probability of the cDNA fragment that occurred in the database by chance. *Subclones selected for subsequent Northern blot analysis.

Table 3.10.12. DNA sequences of cDNA fragments subcloned from gel AI (AP6 and ARP4)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Sequencing				
					Size of insert (bp)	Sequencing primer	Gene homology (Transcript size)	Identities	E value
AI (AP6 & ARP4)	AI1	1100	1110	AI1#5	1110	M13 forward (-20)	*Mus musculus* serine (or cysteine) proteinase inhibitor, clade A, member 3K, Spi2 (1645 bp)	343/356 (96%)	0
						M13 reverse		262/269 (97%)	e-123
	AI18	270	280	AI18#6	300	M13 forward (-20)	*Mus musculus* argininosuccinate lyase, Asl (1589 bp)	184/188 (97%)	6e-84
						M13 reverse		215/217 (99%)	e-110

M13 (-20) forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers were used for sequencing. After sequencing, sequence homology of the identified genes was searched against the known genes and ESTs in GenBank using BLAST. The identities indicate the percentage of the cDNA fragment match with the BLAST search sequence, while the expect value (E) is the parameter to describe the probability of the cDNA fragment that occurred in the database by chance. *Subclones selected for subsequent Northern blot analysis.

Table 3.10.13. DNA sequences of cDNA fragments subcloned from gel AJ (AP6 and ARP14)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Sequencing				
					Size of insert (bp)	Sequencing primer	Gene homology (Transcript size)	Identities	E value
AJ (AP6 &ARP14)	AJ1	1200	1210	AJ1#4	990+ 220	M13 forward (-20)	Mouse carboxylesterase, carboxylesterase (2006 bp)	354/393 (90%)	e-123
				AJ1#5*	990+ 220	M13 reverse		353/379 (93%)	e-127
	AJ2	1100	1110	AJ2#8	1130	M13 forward (-20)	Failed in sequencing		
				AJ2#10*	1130	M13 forward (-20)	*Mus musculus* peroxisomal acyl-coA oxidase, AOX (3778 bp)	481/509 (94%)	0
						M13 reverse		376/393 (95%)	e-170
	AJ9	320	330	AJ9#1	360	M13 forward (-20)	if84h05.x1 Kaestner ngn3 - - subtracted *Mus musculus* cDNA 3' similar to SW:CATA_CAMJE Q59296 CATALASE (280 bp)	206/212 (97%)	1e-93
						M13 reverse	*Mus musculus* suppressor of actin mutations, SAC1 gene (2029 bp)	77/84 (91%)	2e-24

M13 (-20) forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers were used for sequencing. After sequencing, sequence homology of the identified genes was searched against the known genes and ESTs in GenBank using BLAST. The identities indicate the percentage of the cDNA fragment match with the BLAST search sequence, while the expect value (E) is the parameter to describe the probability of the cDNA fragment that occurred in the database by chance. *Subclones selected for subsequent Northern blot analysis.

Table 3.10.14. DNA sequences of cDNA fragments subcloned from AL (AP7 and ARP15)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Sequencing				
					Size of insert (bp)	Sequencing primer	Gene homology (Transcript size)	Identities	E value
AL (AP7 & ARP15)	AL2	350	370	AL2#8	390	M13 forward (-20)	*Mus musculus* hydroxysteroid (17-beta) dehydrogenase 11, HSD17b11 (1713 bp)	308/308 (100%)	e-171
				AL2#9	390	M13 reverse	Failed in sequencing		
	AL3	345	370	AL3#3*	380	M13 forward (-20)	*Mus musculus* hydroxysteroid (17-beta) dehydrogenase 11, HSD17b11 (1713 bp)	306/307 (99%)	e-166
						M13 reverse		304/308 (98%)	e-155

M13 (-20) forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers were used for sequencing. After sequencing, sequence homology of the identified genes was searched against the known genes and ESTs in GenBank using BLAST. The identities indicate the percentage of the cDNA fragment match with the BLAST search sequence, while the expect value (E) is the parameter to describe the probability of the cDNA fragment that occurred in the database by chance. *Subclones selected for subsequent Northern blot analysis.

Table 3.10.15. DNA sequences of cDNA fragments subcloned from gel AO (AP5 and ARP10)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Sequencing				
					Size of insert (bp)	Sequencing primer	Gene homology (Transcript size)	Identities	E value
AO (AP5 & ARP10)	AO1	700	700	AO1#2*	720	M13 forward (-20)	Mouse adipose differentiation related protein, ADRP (1680 bp)	530/564 (93%)	0
				AO1#5	720	M13 reverse	*Mus musculus* carnitine O-octanoyltransferase, Crot (2844 bp)	461/498 (92%)	e-152
	AO2	695	710	AO2#6	760	M13 forward (-20)	*Mus musculus* ribonuclease, RNase A family 4, Rnase4 (1412 bp)	458/471 (97%)	0
						M13 reverse		435/454 (95%)	0
				AO2#8*	740	M13 forward (-20)	Failed in sequencing		
						M13 reverse	*Mus musculus* carnitine O-octanoyltransferase, Crot (2844 bp)	319/330 (96%)	e-160
	AO8	400	410	AO8#2	400	M13 forward (-20)	*Mus musculus* ribonuclease, RNase A family 4, Rnase4 (1412 bp)	294/315 (93%)	e-121

M13 (-20) forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers were used for sequencing. After sequencing, sequence homology of the identified genes was searched against the known genes and ESTs in GenBank using BLAST. The identities indicate the percentage of the cDNA fragment match with the BLAST search sequence, while the expect value (E) is the parameter to describe the probability of the cDNA fragment that occurred in the database by chance. *Subclones selected for subsequent Northern blot analysis.

Table 3.10.16. DNA sequences of cDNA fragments subcloned from gel AP (AP12 and ARP2)

Gel	FluoroDD fragment I.D.	FluoroDD fragment size (bp)	Reamplified fragment size (bp)	Subclone I.D.	Sequencing				
					Size of insert (bp)	Sequencing primer	Gene homology (Transcript size)	Identities	E value
AP (AP12 & ARP2)	AP4	680	690	AP4#4	720	M13 forward (-20)	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	113/123 (91%)	7e-30
						M13 reverse		221/232 (95%)	7e-95

M13 (-20) forward (5'-GTAAAACGACGGCCAG-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3') primers were used for sequencing. After sequencing, sequence homology of the identified genes was searched against the known genes and ESTs in GenBank using BLAST. The identities indicate the percentage of the cDNA fragment match with the BLAST search sequence, while the expect value (E) is the parameter to describe the probability of the cDNA fragment that occurred in the database by chance. *Subclones selected for subsequent Northern blot analysis.

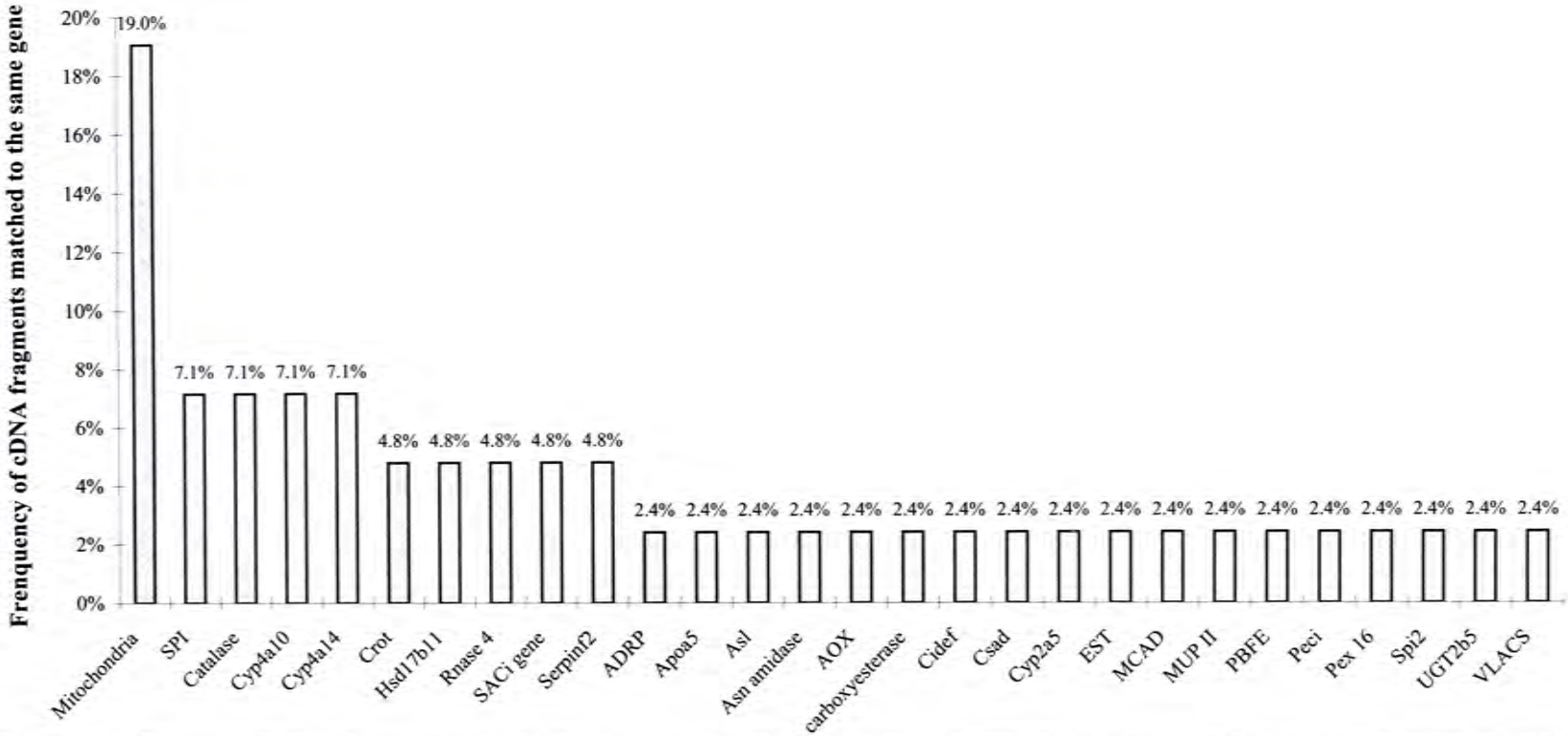

Figure 3.10.1. Frequency of cDNA fragments matched to the same gene (no. of cDNA / total no. of cDNA fragments successfully sequenced X 100%). Total 26 differents genes sequenced, which are PPARα-dependent and Wy-14, 643 responsive, were obtained. ADRP, adipose differentiation related protein; Apoa5, apolipoprotein A-V; Asl, argininosuccinate lyase; Asn amidase, N-terminal Asn amidase; AOX, peroxisomal acyl-CoA oxidase; Carboxyesterase, carboxyesterase; Catalase, catalase; Crot, carnitine O-octanoyltransferase; Cidef, cell death-inducing DNA fragmentation factor, alpha subunit-like effector B; Csad, cysteine sulfinic acid decarboxylase; Cyp2a5, cytochorme P450 2a5; Cyp4a10, cytochorme P450 4a10; Cyp4a14, cytochorme P450 4a14; Peci, peroxisomal delta3, delta2-enoyl-Coenzyme A isomerase; EST, mouse EST; Hsd17b11, hydroxysteroid (17-beta) dehydrogenase 11; MCAD, medium chain acetyl-Coenzyme A dehydrogenase; Mitochondria, mouse complete mitochondrial genome; MUP II, major urinary protein II; PBFE, peroxisomal bifunctional enzyme; Pex 16, peroxisome biogenesis factor 16; RNase 4, ribonuclease, RNase A family 4; SPI, serine (or cysteine) proteinase inhibitor; SAC1 gene, suppressor of actin mutations; UGT2b5, UDP-glucuronosyltransferase; and VLACS, very-long-chain acyl-CoA synthetase.

(4.8%), Hsd17β11 (4.8%), Rnase 4 (4.8%), SAC1 gene (4.8%) and Serpinf2 (4.8%) > ADRP (2.4%), Apoa5 (2.4%), Asl (2.4%), Asn amidase (2.4%), AOX (2.4%), Carboxyesterase (2.4%), Cidef (2.4%), Csad (2.4%), Cyp2a5 (2.4%), EST (2.4%), MCAD (2.4%), MUP II (2.4%), PBFE (2.4%), Peci (2.4%), Pex16 (2.4%), Spi2 (2.4%), UGT2b5 (2.4%) and VLACS (2.4%).

3.11 Northern blot analysis of sequenced cDNA fragments

A total of seventeen subcloned cDNA fragments were confirmed. They included AA1#2 [mouse peroxisomal delta3, delta2-enoyl-Coenzyme A isomerase (Peci) (Figure 3.11.1)]; AA4#9 [mouse apolipoprotein A-V (Apoa5) (Figure 3.11.2)]; AA10#1 [cysteine sulfinic acid decarboxylase (Csad) (Figure 3.11.3)]; AA12#4 [mouse acetyl-Coenzyme A dehydrogenase, medium chain (MCAD) (Figure 3.11.4)]; AB7#2 [mouse UDP-glucuronosyltransferase 2 family member 5, (UDP2b5) (Figure 3.11.5)]; AB22#9 [peroxisome biogenesis factor 16 (Pex 16) (Figure 3.11.6)]; AB25#6 [mouse cytochrome P450 4a14 (Cyp4a14) (Figure 3.11.7)]; AC1#2 [mouse serine protease inhibitor, clade A, member 1a (SPI) (Figure 3.11.8)]; AC2#2 [mouse peroxisomal bifunctional enzyme (PBFE) (Figure 3.11.9)]; AD8#2 [mouse cytochrome P450 4a10 (Cyp4a10) (Figure 3.11.10)]; AF1#8 [very-long-chain acyl-CoA synthetase (VLACS) (Figure 3.11.11)]; AF25#7 [mouse major urinary protein II (MUP II) (Figure 3.11.12)]; AJ1#5 [mouse carboxyesterase (carboxyesterase) (Figure 3.11.13)]; AJ2#10 [mouse peroxisomal acyl-CoA oxidase (AOX) (Figure 3.11.14)]; AL3#3 [mouse hydroxysteroid (17-beta) dehydrogenase 11, (Hsd17β11) (Figure 3.11.15)]; AO1#2 [mouse adipose differentiation related protein (ADRP) (Figure 3.11.16)] and AO2#8 [mouse carnitine O-octanoyltransferase (Crot) (Figure 3.11.17)]. The results of the Northern blot analysis were then quantified by MultiAnalyst and the

fold changes of Wy-14,643 / Control of PPARα (+/+) and PPARα (-/-) mice were calculated.

For loading normalization, the RNA gel photo for each Northern blot analysis was showed. Housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was not used for normalization of northern blot analysis as its expression was up-regulated in the Wy-14,643 treated PPARα (+/+) mice after 11 months treatment (Figure 3.11.18) and this observation was also mentioned in previous studie (Anderson *et al.*, 1999).

Some of the subcloned cDNA fragments could not be confirmed by Northern blot analysis as no differential display pattern was observed. They included AA7#3, AB19#10 and AP4#4 [mouse complete mitochondrial genome (mitochondria) (Figure 3.11.19)]; AC1#1 [mouse serine (or cysteine) proteinase inhibitor, clade F, member 2 (Serpinf2)]; AF21#5 [mouse cell death-inducing DNA fragmentation factor, alpha subunit-like effector B (Cidef)]; AJ9#1 [mouse suppressor of actin mutations (SAC1 gene)] and AI18#6 [mouse argininosuccinate lyase (Asl)]. In addition, some of the subcloned cDNA fragments did not give any signal in the Northern blot analysis, including AB29#7 [mouse catalase]; AD6#4 [mouse N-terminal Asn amidase]; AH1#1 [mouse expressed sequence tags (EST) (Figure 3.11.20)] and AO2#6 [mouse ribonuclease, RNase A family 4 (RNase4)].

Up-regulation in PPARα (+/+) mice fed with the 0.1% Wy-14,643 diet was observed in subcloned cDNA fragments AA1#2 [mouse peroxisomal delta3, delta2-enoyl-Coenzyme A isomerase (Peci) (3-fold) (Figure 3.11.1) (Table3.11.1)]; AA10#1 [cysteine sulfinic acid decarboxylase (Csad) (20-fold) (Figure 3.11.3) (Table 3.11.1)]; AA12#4 [mouse acetyl-Coenzyme A dehydrogenase, medium chain (MCAD) (3.35-fold) (Figure 3.11.4) (Table 3.11.1)]; AB22#9 [peroxisome biogenesis factor 16 (Pex

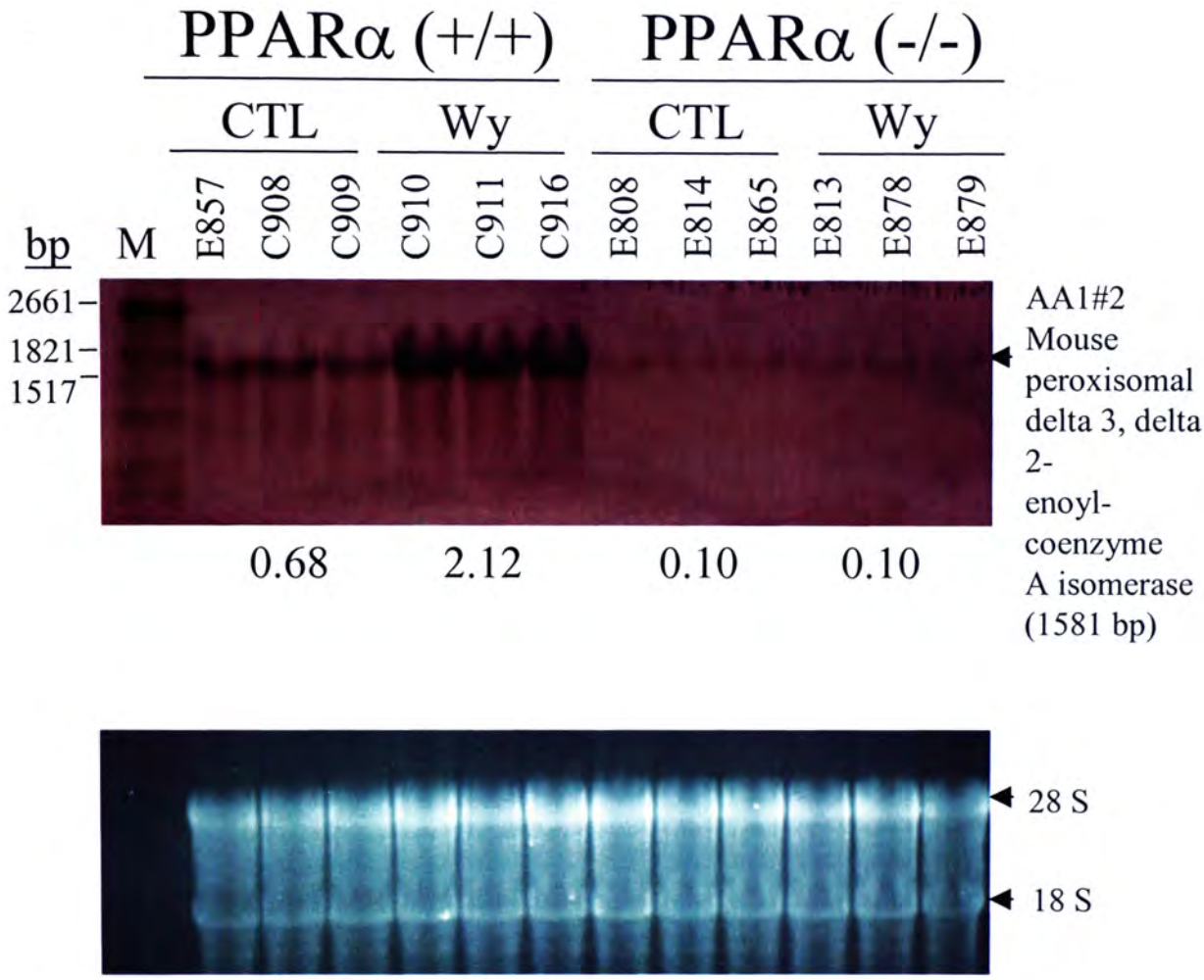

Figure 3.11.1. Confirmation of fluoroDD expression pattern of subclone AA1#2 (peroxisomal delta 3, delta 2-enoyl-coenzyme A isomerase) by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a PCR DIG-labeled cDNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

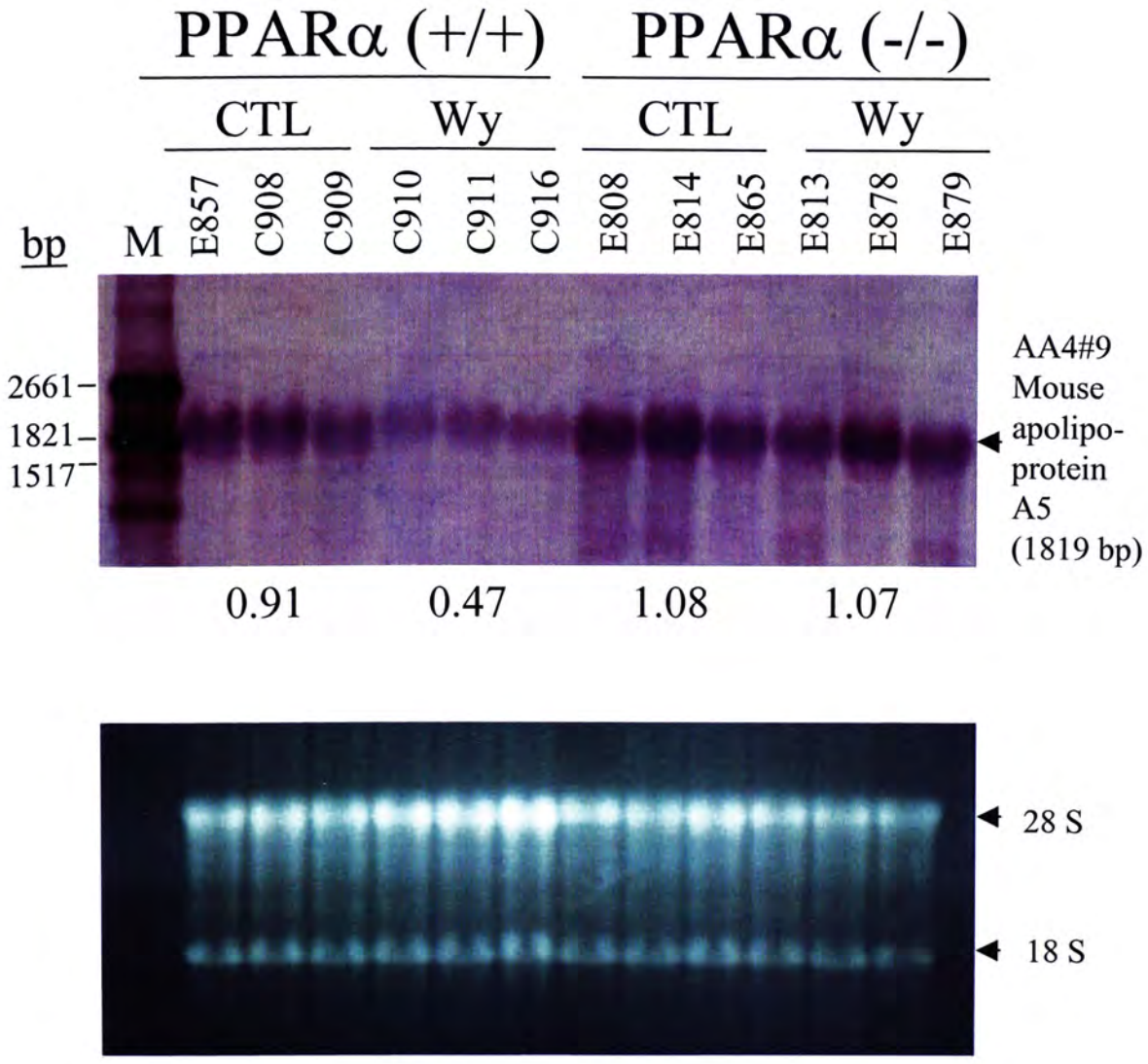

Figure 3.11.2. Confirmation of fluoroDD expression pattern of subclone AA4#9 (apolipoprotein A-V) by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a PCR DIG-labeled cDNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

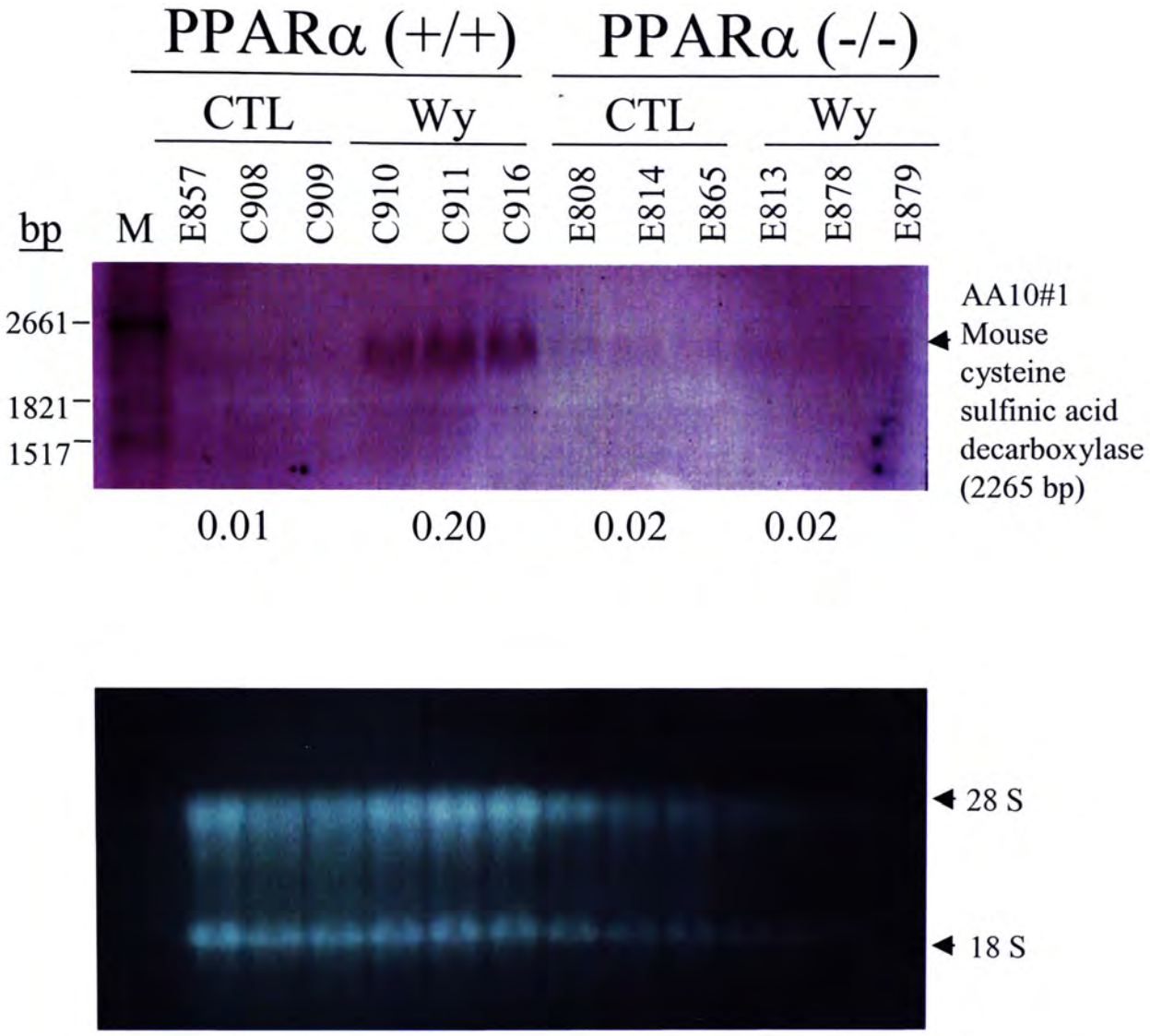

Figure 3.11.3. Confirmation of fluoroDD expression pattern of subclone AA10#1 (cysteine sulfinic acid decarboxylase) by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

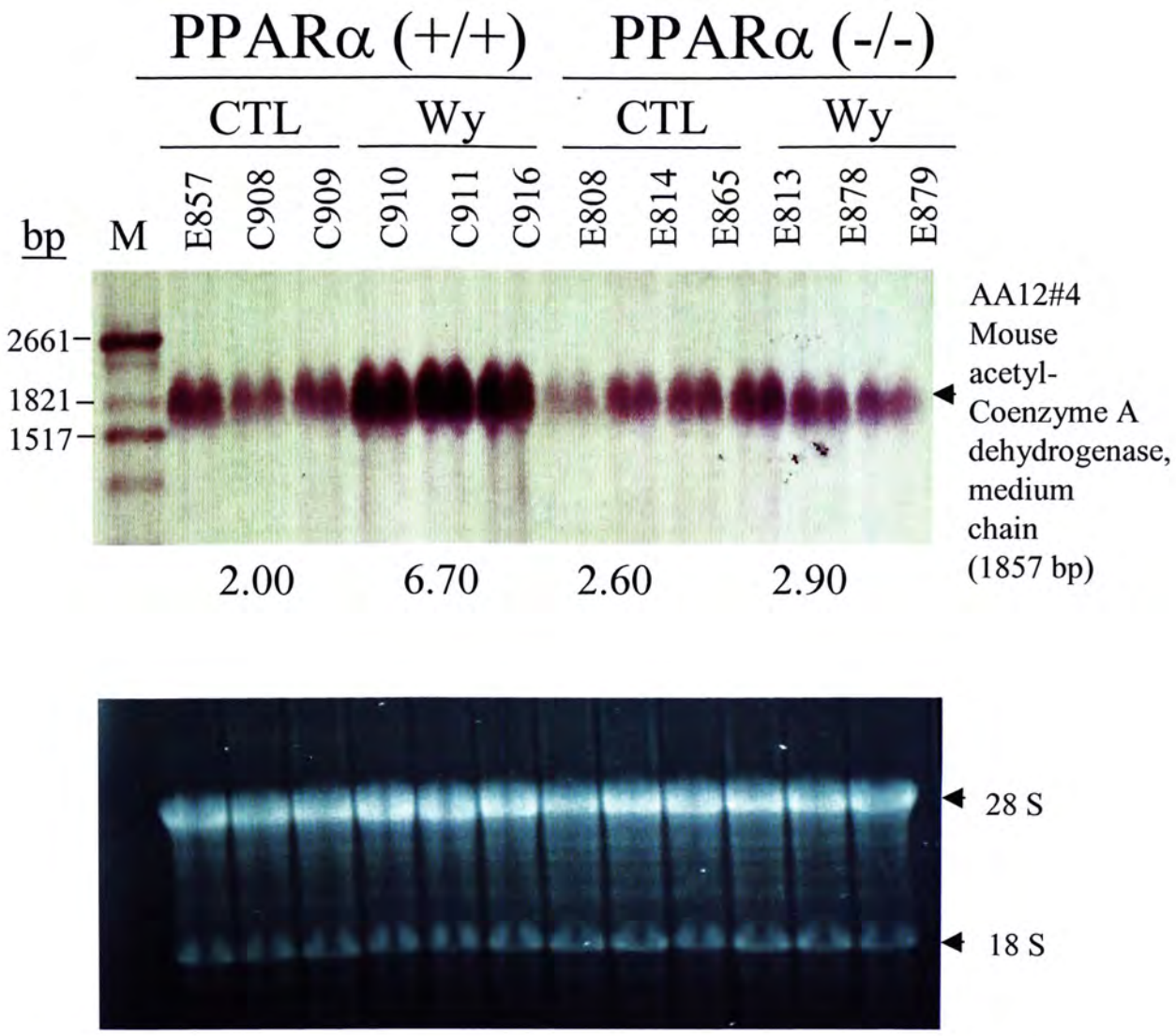

Figure 3.11.4. Confirmation of fluoroDD expression pattern of subclone AA12#4 (acetyl-Coenzyme A dehydrogenase, medium chain) by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

Table 3.11.1. Summary of Northern blot analysis of cDNA fragments subcloned from gel AA (AP1 and ARP2)

Gel	Subclone I.D.	Differential display pattern				Gene homology (Transcript size)	Northern blot analysis densitometry reading (arbitary unit)					
		PPARα (+/+)		PPARα(-/-)			PPARα (+/+)			PPARα (-/-)		
		CTL	Wy	CTL	Wy		CTL	Wy	Fold change (Wy/CTL)	CTL	Wy	Fold change (Wy/CTL)
AA (AP1 & ARP2)	AA1#2	-	+	-	-	*Mus musculus* peroxisomal delta 3, delta 2-enoyl-coenzyme A isomerase, Peci (1581 bp)	0.68 ± 0.13 ++	2.12 ± 0.17 +++	3.12	0.10 ± 0.01 -	0.10 ± 0.01 -	1.00
	AA4#9	++	+	++	++	*Mus musculus* apolipoprotein A-V, Apoa5 (1819 bp)	0.91 ± 0.17 ++	0.47 ± 0.02 +	0.52	1.08 ± 0.11 ++	1.07 ± 0.41 ++	0.99
	AA10#1	+	++	+	+	*Mus musculus* cysteine sulfinic acid decarboxylase, Csad (2265 bp)	0.01 ± 0.01 -	0.20 ± 0.03 +	20.00	0.02 ± 0.01 -	0.02 ± 0.01 -	1.00
	AA12#4	+	++	+	+	*Mus musculus* acetyl-coenzyme A dehydrogenase, medium chain, MCAD (1857 bp)	2.00 ± 0.89 +	6.70 ± 1.68 ++	3.35	2.60 ± 0.59 +	2.90 ± 0.44 +	1.12

Values represent mean ± S.D. '+' represents the presence of a band (cDNA) on fluoroDD gel or Northern blot, while '-' represents the corresponding band (cDNA) was not observed on the fluoroDD gel or Northern blot. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. The signal intensity of a band on Northern blot was quantified by MultiAnalyst.

16) (4-fold) (Figure 3.11.6) (Table 3.11.2)]; AB25#6 [mouse cytochrome P450 4a14 (Cyp4a14) (105-fold) (Figure 3.11.7) (Table 3.11.2)]; AC2#2 [mouse peroxisomal bifunctional enzyme (PBFE) (20-fold) (Figure 3.11.9) (Table 3.11.3)]; AD8#2 [mouse cytochrome P450 4a10 (Cyp4a10) (115-fold) (Figure 3.11.10) (Table 3.11.3)]; AF1#8 [very-long-chain acyl-CoA synthetase (VLACS) (2-fold) (Figure 3.11.20) (Table 3.11.4)]; AJ2#10 [mouse peroxisomal acyl-CoA oxidase (AOX) (10-fold) (Figure 3.11.14) (Table 3.11.4)]; AL3#3 [mouse hydroxysteroid (17-beta) dehydrogenase 11, (Hsd17β11) (10-fold) (Figure 3.11.15) (Table 3.11.5)]; AO1#2 [mouse adipose differentiation related protein (ADRP) (16-fold) (Figure 3.11.16) (Table 3.11.5)] and AO2#8 [mouse carnitine O-octanoyltransferase (Crot) (4-fold) (Figure 3.11.17) (Table 3.11.5)] were confirmed.

Subcloned cDNA fragments AA4#9 [mouse apolipoprotein A-V (Apoa5) (0.5-fold) (Figure 3.11.2) (Table 3.11.1)]; AB7#2 [mouse UDP-glucuronosyltransferase 2 family member 5, (UDP2b5) (0.5-fold) (Figure 3.11.5) (Table 3.11.2)]; AC1#2 [mouse serine protease inhibitor, clade A, member 1a (SPI) (0.24-fold) (Figure 3.11.8) (Table 3.11.3)]; AF25#7 [mouse major urinary protein II (MUP II) (0.08-fold) (Figure 3.11.12) (Table 3.11.4)] and AJ1#5 [mouse carboxyesterase (carboxyesterase) (0.08-fold) (Figure 3.11.13) (Table 3.11.4)] were confirmed to be down-regulated by 0.1% Wy-14,643 and PPARα-dependent.

Subcloned cDNA fragments AB7#2 [mouse UDP-glucuronosyltransferase 2 family member 5, (UDP2b5)], AC1#2 [mouse serine protease inhibitor, clade A, member 1a (SPI)], AD8#2 [mouse cytochrome P450 4a10 (Cyp4a10)] and AF25#7 [mouse major urinary protein II (MUP II)] showed several bands in the Northern blot analysis. The different bands might be different isoforms of the genes. Subcloned cDNA fragments AB7#2 [mouse UDP-glucuronosyltransferase 2 family member 5,

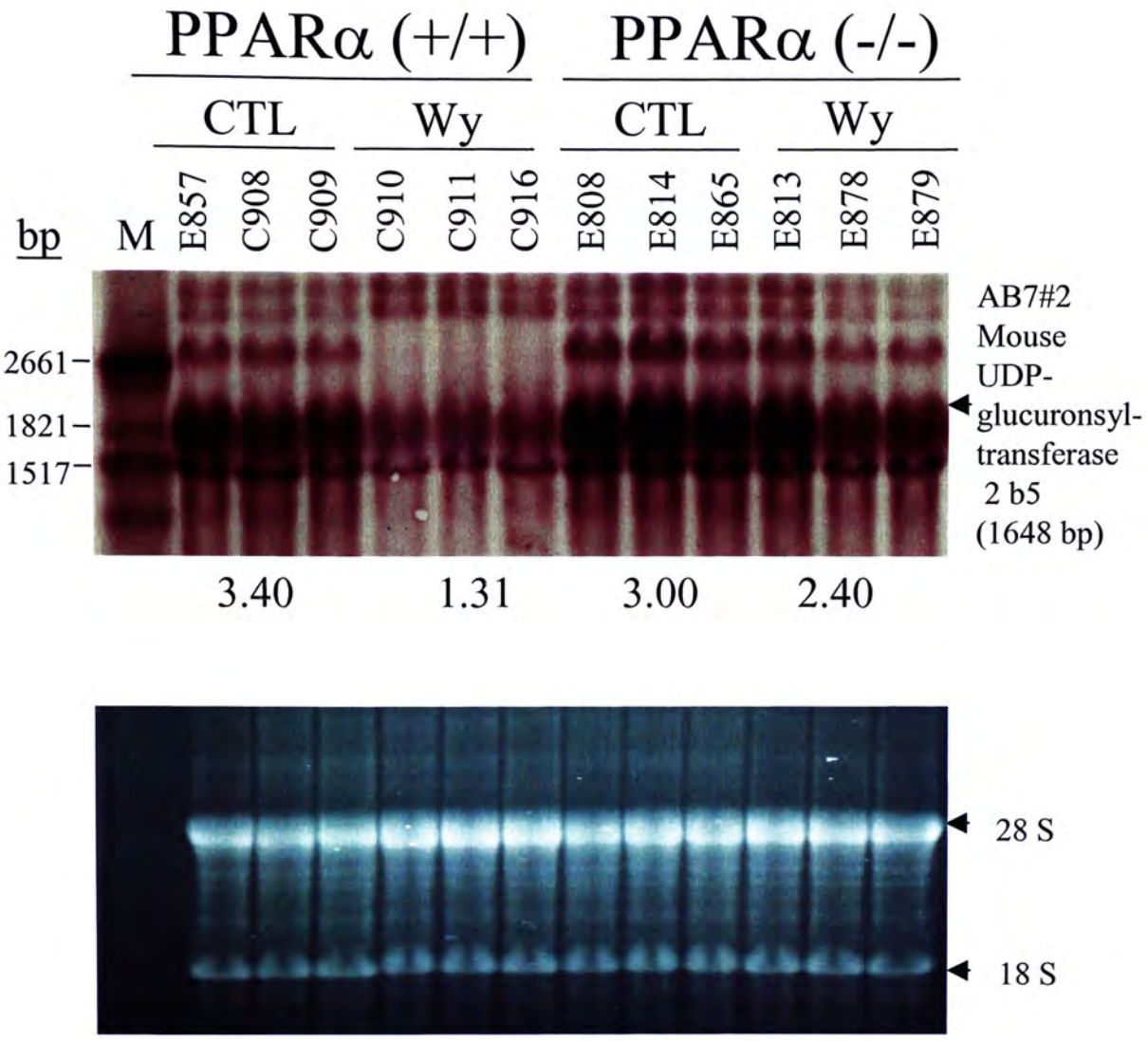

Figure 3.11.5. Confirmation of fluoroDD expression pattern of subclone AB7#2 (UDP-glucuronsyltransferase 2b5) by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a PCR DIG-labeled cDNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

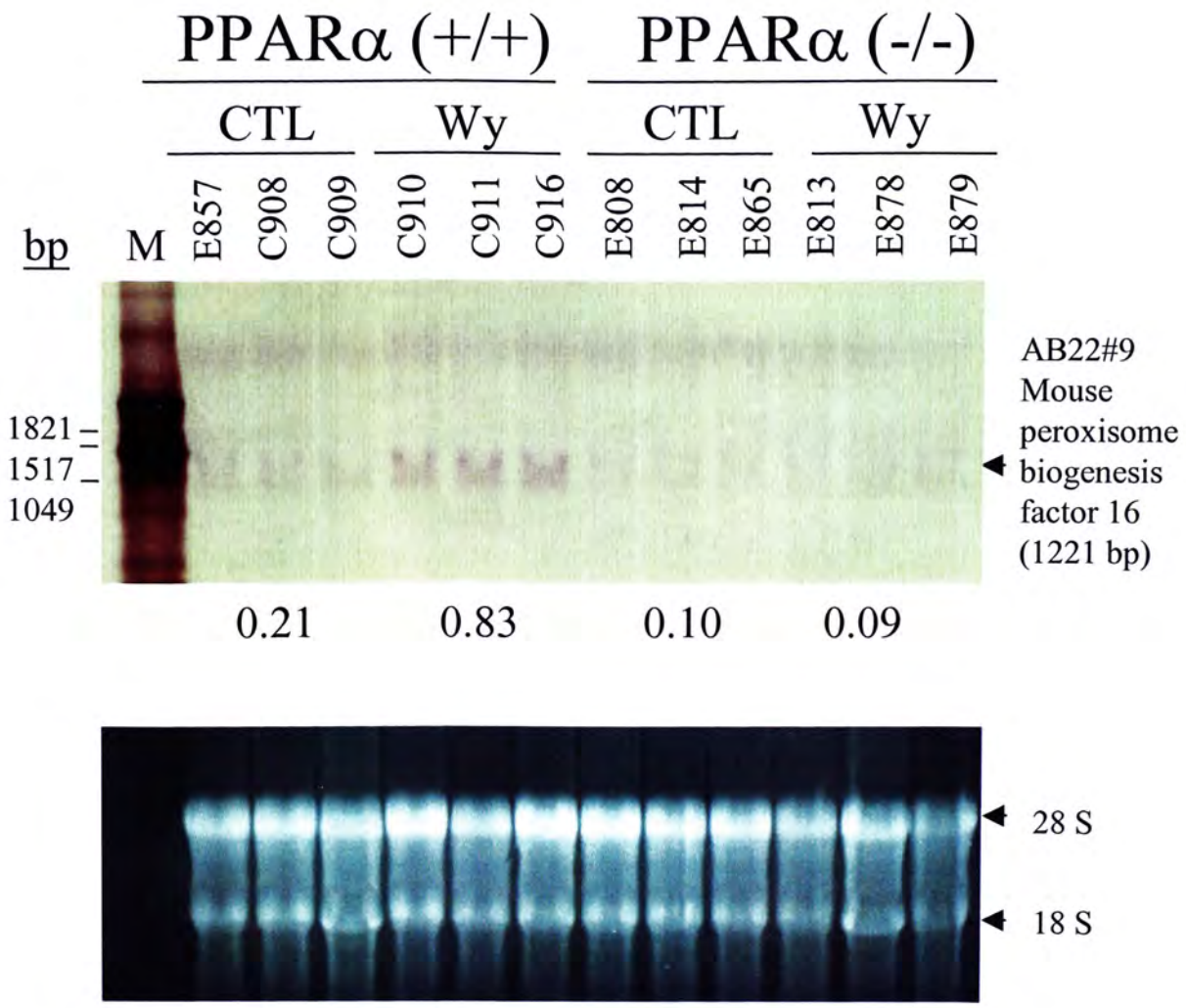

Figure 3.11.6. Confirmation of fluoroDD expression pattern of subclone AB22#9 (peroxisome biogenesis factor 16) by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a PCR DIG-labeled cDNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

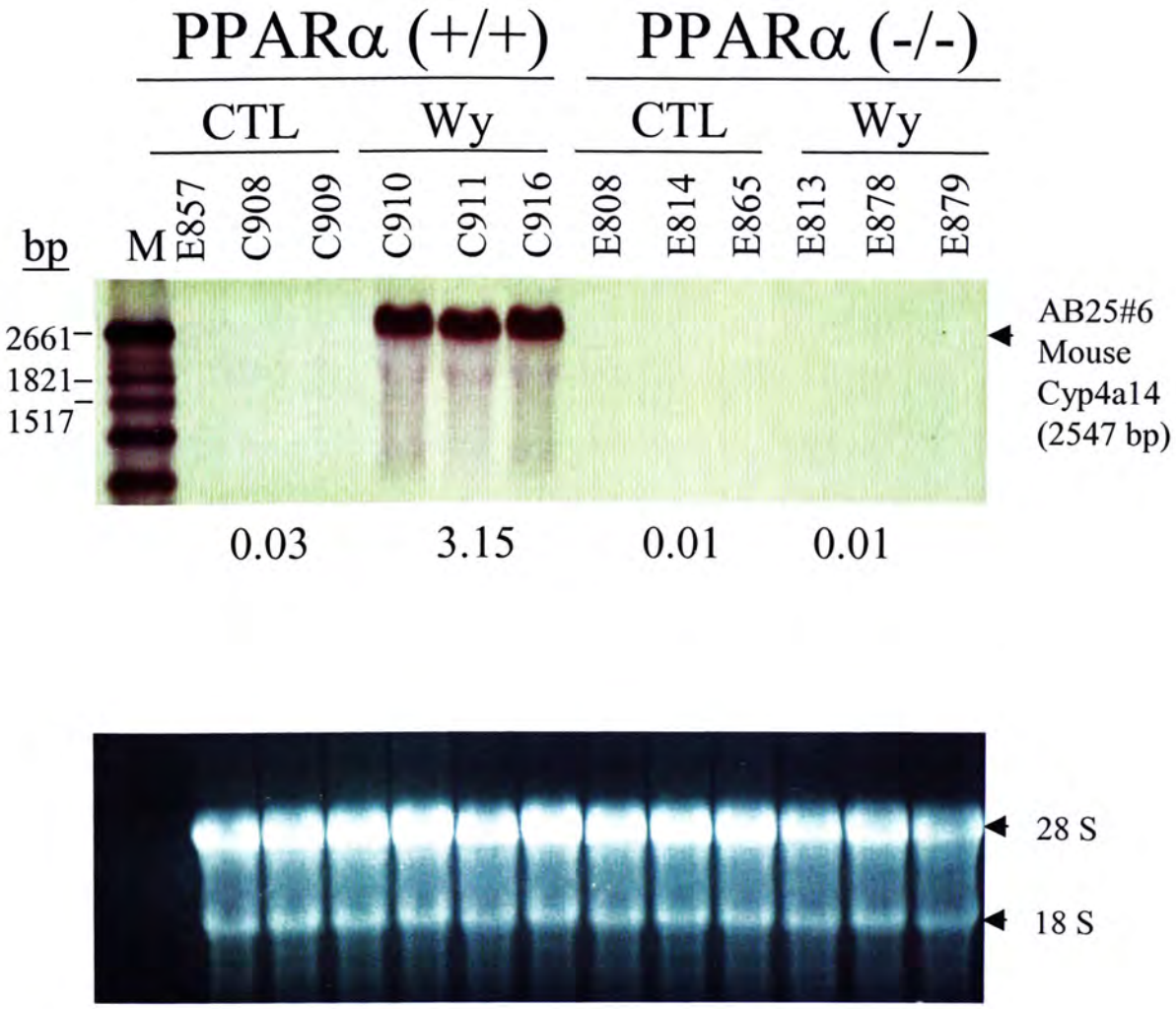

Figure 3.11.7. Confirmation of fluoroDD expression pattern of subclone AB25#6 (cytochrome P450, family 4, subfamily a, polypeptide 14) by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a PCR DIG-labeled cDNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

Table 3.11.2. Summary of Northern blot analysis of cDNA fragments subcloned from gel AB (AP3 and ARP3)

Gel	Subclone I.D.	Differential display pattern				Gene homology (Transcript size)	Northern blot analysis densitometry reading (arbitary unit)					
		PPARα (+/+)		PPARα(-/-)			PPARα (+/+)			PPARα (-/-)		
		CTL	Wy	CTL	Wy		CTL	Wy	Fold change (Wy/CTL)	CTL	Wy	Fold change (Wy/CTL)
AB (AP3 & ARP3)	AB7#2	+	++	+	+	Mouse UDP-glucuronosyltransferase 2 family, member 5, UGT2b5 (1648 bp)	3.40 ± 0.53 ++	1.31 ± 0.08 +	0.39	3.00 ± 0.61 ++	2.40 ± 0.21 ++	0.80
	AB22#9	+	++	+	+	*Mus musculus* peroxisome biogenesis factor 16, Pex16 (1221 bp)	0.21 ± 0.05 +	0.83 ± 0.06 ++	3.95	0.10 ± 0.01 +	0.09 ± 0.05 +	0.90
	AB25#6	-	+	-	-	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 14, Cyp4a14 (2547 bp)	0.03 ± 0.02 -	3.15 ± 0.64 ++	105.00	0.01 ± 0.01 -	0.01 ± 0.01 -	1.00

Values represent mean ± S.D. '+' represents the presence of a band (cDNA) on fluoroDD gel or Northern blot, while '-' represents the corresponding band (cDNA) was not observed on the fluoroDD gel or Northern blot. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. The signal intensity of a band on Northern blot was quantified by MultiAnalyst.

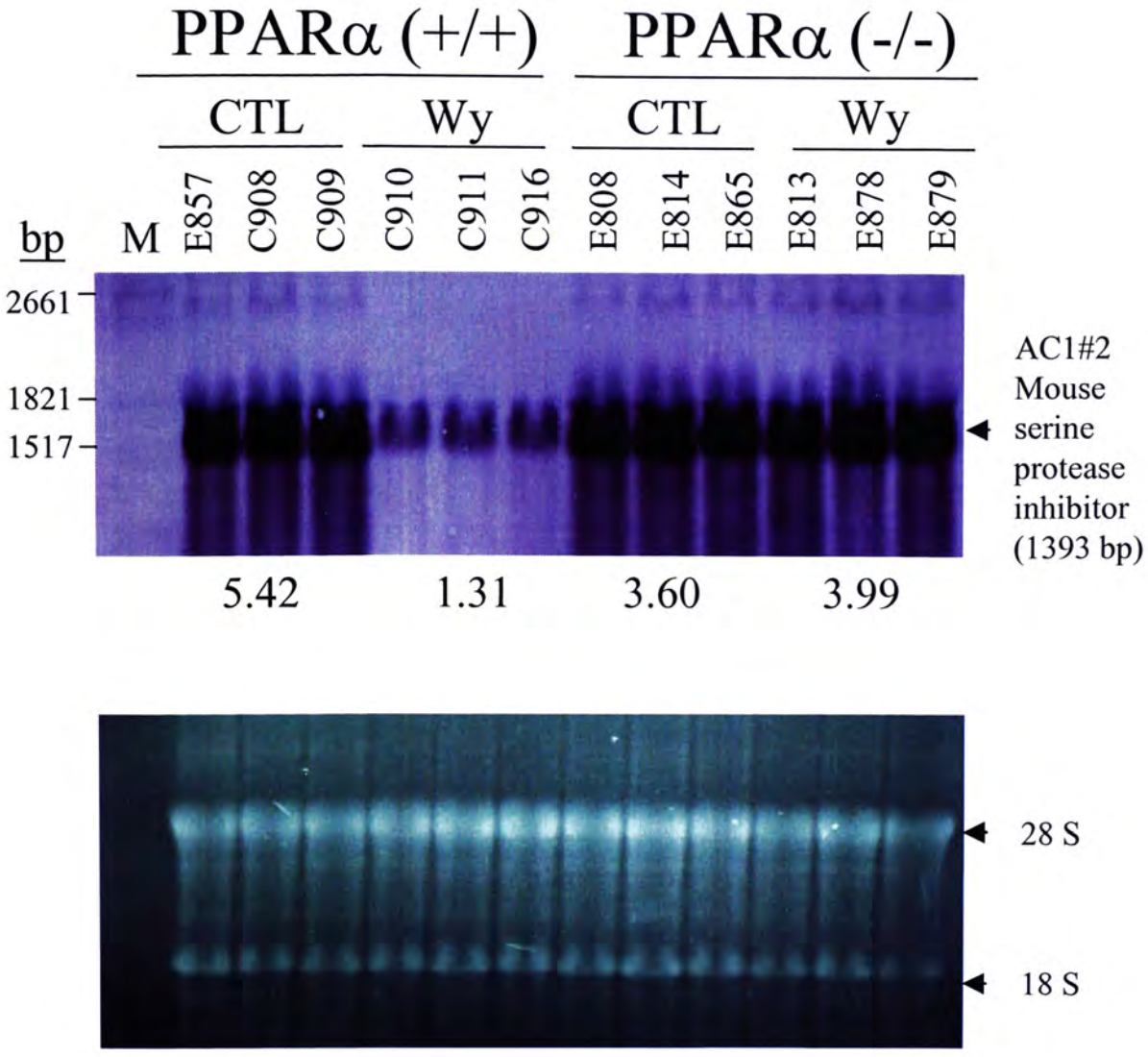

Figure 3.11.8. Confirmation of fluoroDD expression pattern of subclone AC1#2 (serine protease inhibitor) by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

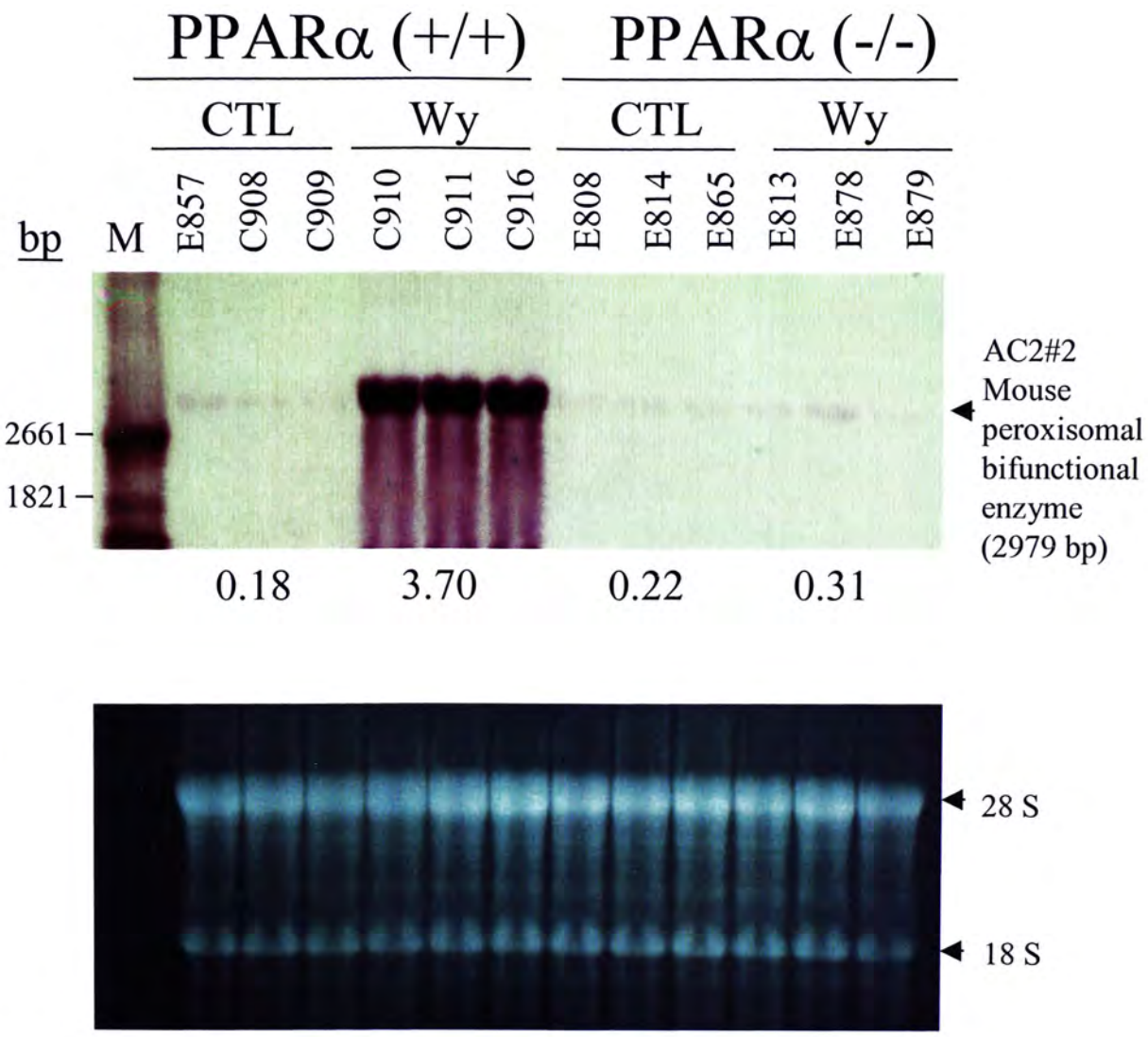

Figure 3.11.9. Confirmation of fluoroDD expression pattern of subclone AC2#2 (peroxisomal bifunctional enzyme) by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

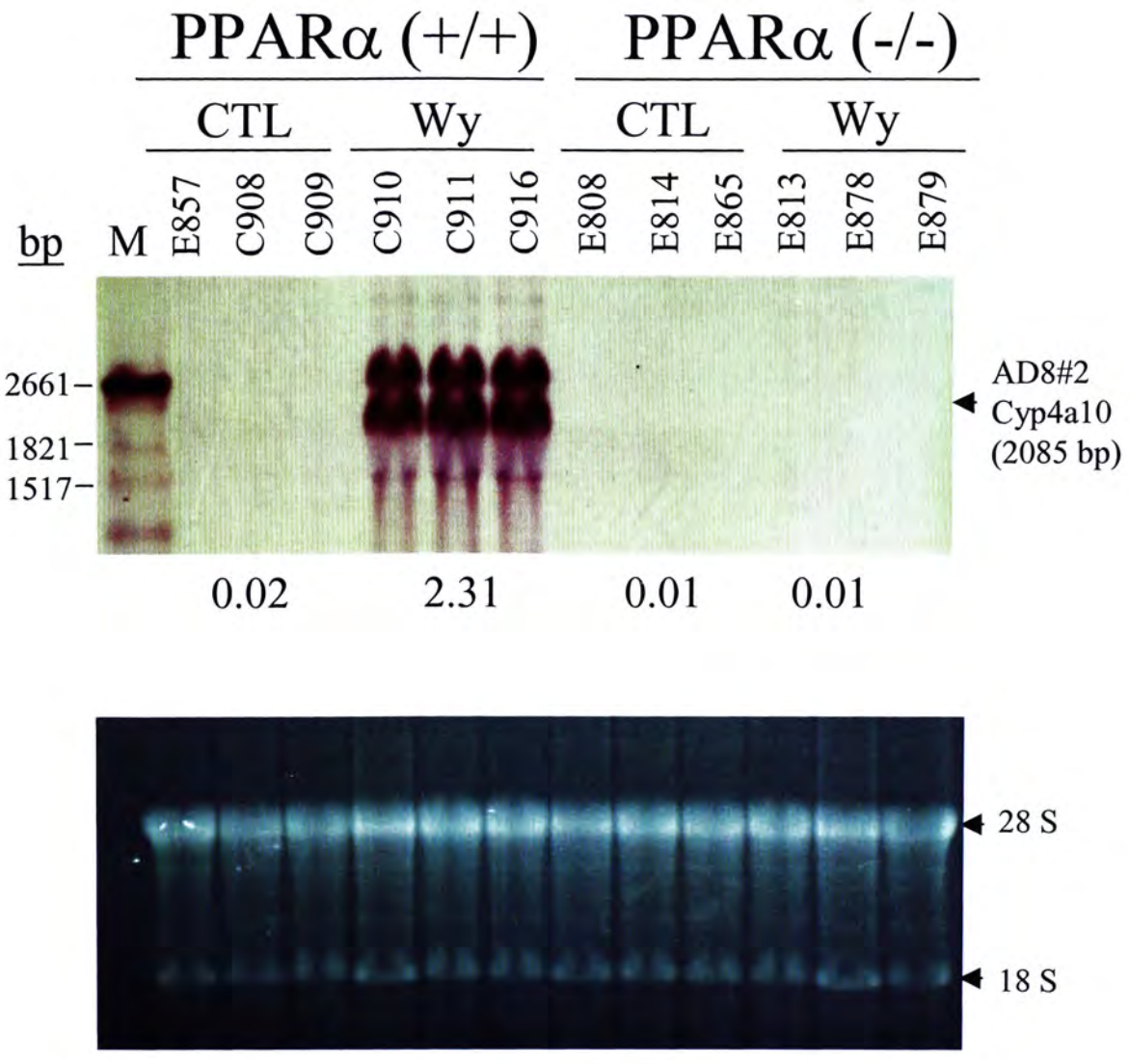

Figure 3.11.10. Confirmation of fluoroDD expression pattern of subclone AD8#2 (cytochrome P450, family 4, subfamily a, polypeptide 10) by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

Table 3.11.3. Summary of Northern blot analysis of cDNA fragments subcloned from gels AC (AP2 and ARP19) and AD (AP2 and ARP18)

Gel	Subclone I.D.	Differential display pattern				Gene homology (Transcript size)	Northern blot analysis densitometry reading (arbitary unit)					
		PPARα (+/+)		PPARα(-/-)			PPARα (+/+)			PPARα (-/-)		
		CTL	Wy	CTL	Wy		CTL	Wy	Fold change (Wy/CTL)	CTL	Wy	Fold change (Wy/CTL)
AC (AP2 & ARP19)	AC1#2	+	-	+	+	*Mus musculus* serine (or cysteine) proteinase inhibitor, SPI (1393 bp)	5.42 ± 0.68	1.31 ± 0.15	0.24	3.60 ± 0.72	3.99 ± 0.68	1.11
							+++	+		+++	+++	
	AC2#2	-	+	-	-	*Mus musculus* peroxisomal bifunctional enzyme (PBE) (PBFE) [includes: enoyl-CoA hydratase (EC 4.2.1.17); 3,2-trans_enoyl-CoA isomerase (EC 5.3.3.8); 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35)], PBFE (2979 bp)	0.18 ± 0.08	3.70 ± 0.34	20.56	0.22 ± 0.07	0.31 ± 0.06	1.41
							+	+++		+	+	
AD (AP2 & ARP18)	AD8#2	+	++	+	+	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 10, Cyp4a10 (2085 bp)	0.02 ± 0.02	2.31 ± 0.18	115.50	0.01 ± 0.01	0.01 ± 0.00	1.00
							-	+++		-	-	

Values represent mean ± S.D. '+' represents the presence of a band (cDNA) on fluoroDD gel or Northern blot, while '-' represents the corresponding band (cDNA) was not observed on the fluoroDD gel or Northern blot. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. The signal intensity of a band on Northern blot was quantified by MultiAnalyst.

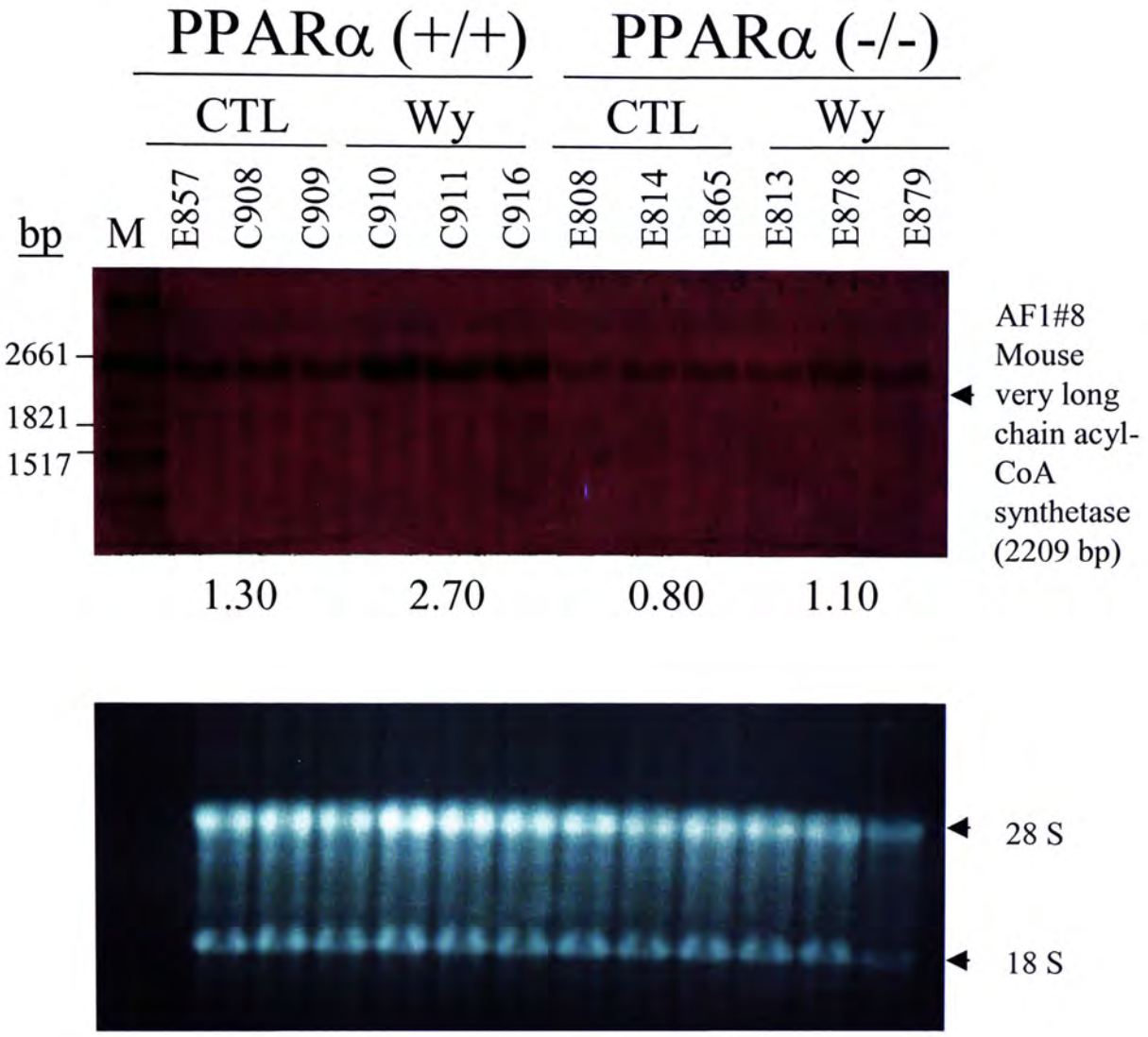

Figure 3.11.11. Confirmation of fluoroDD expression pattern of subclone AF1#8 (very long chain acyl-CoA synthetase) by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a PCR DIG-labeled cDNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

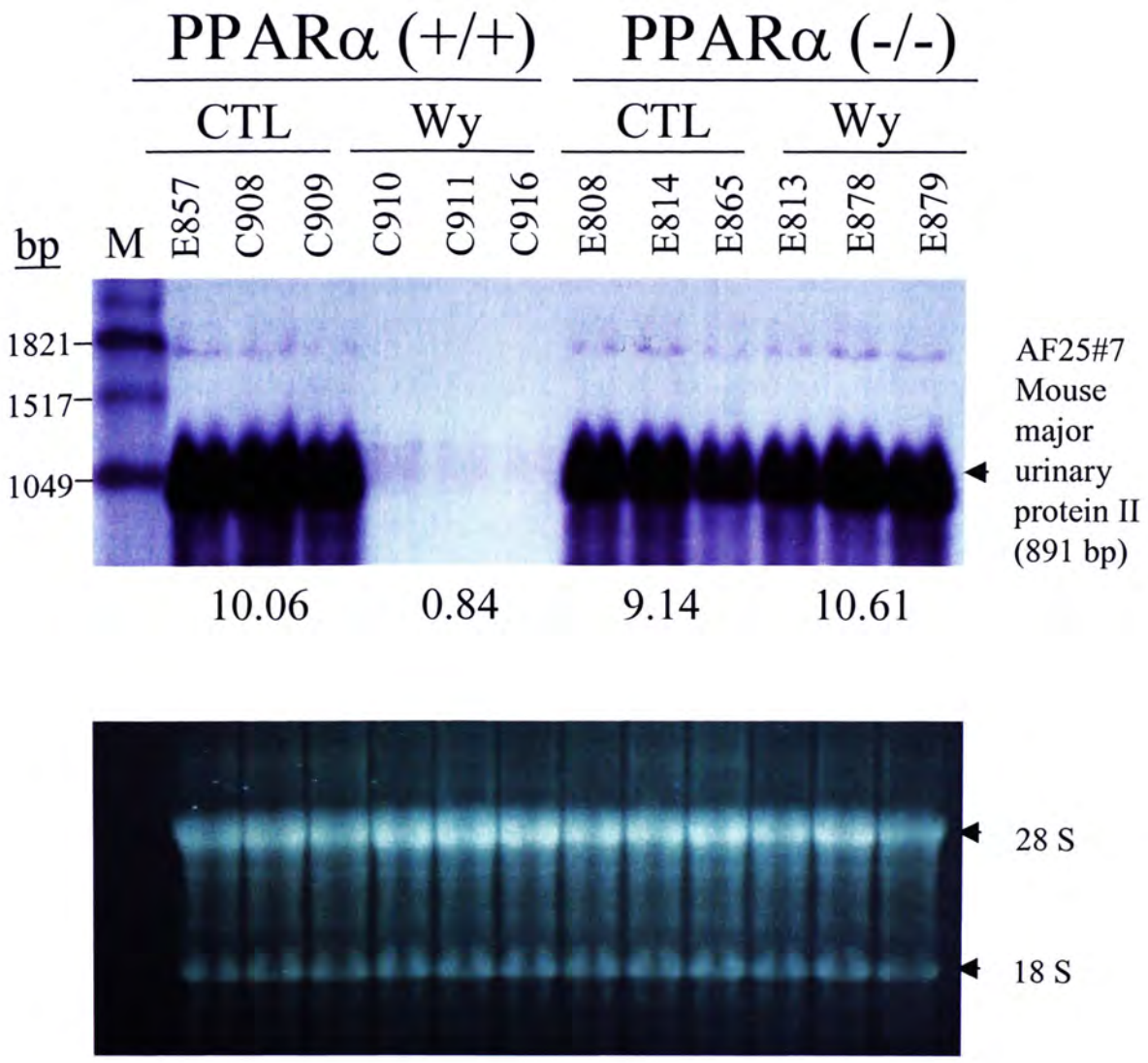

Figure 3.11.12. Confirmation of fluoroDD expression pattern of subclone AF25#7 (mouse major urinary protein II) by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

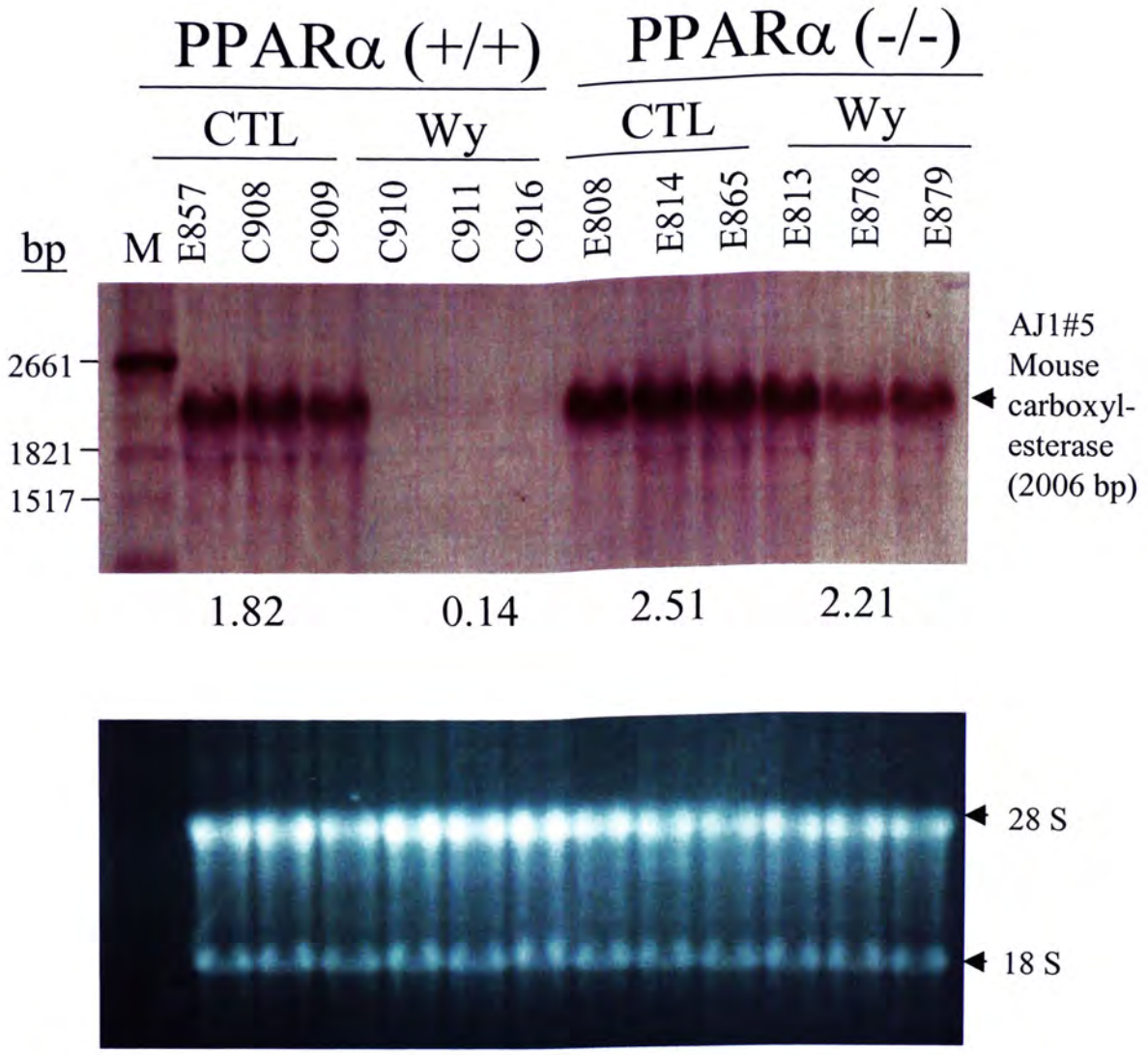

Figure 3.11.13. Confirmation of fluoroDD expression pattern of subclone AJ1#5 (carboxylesterase) by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

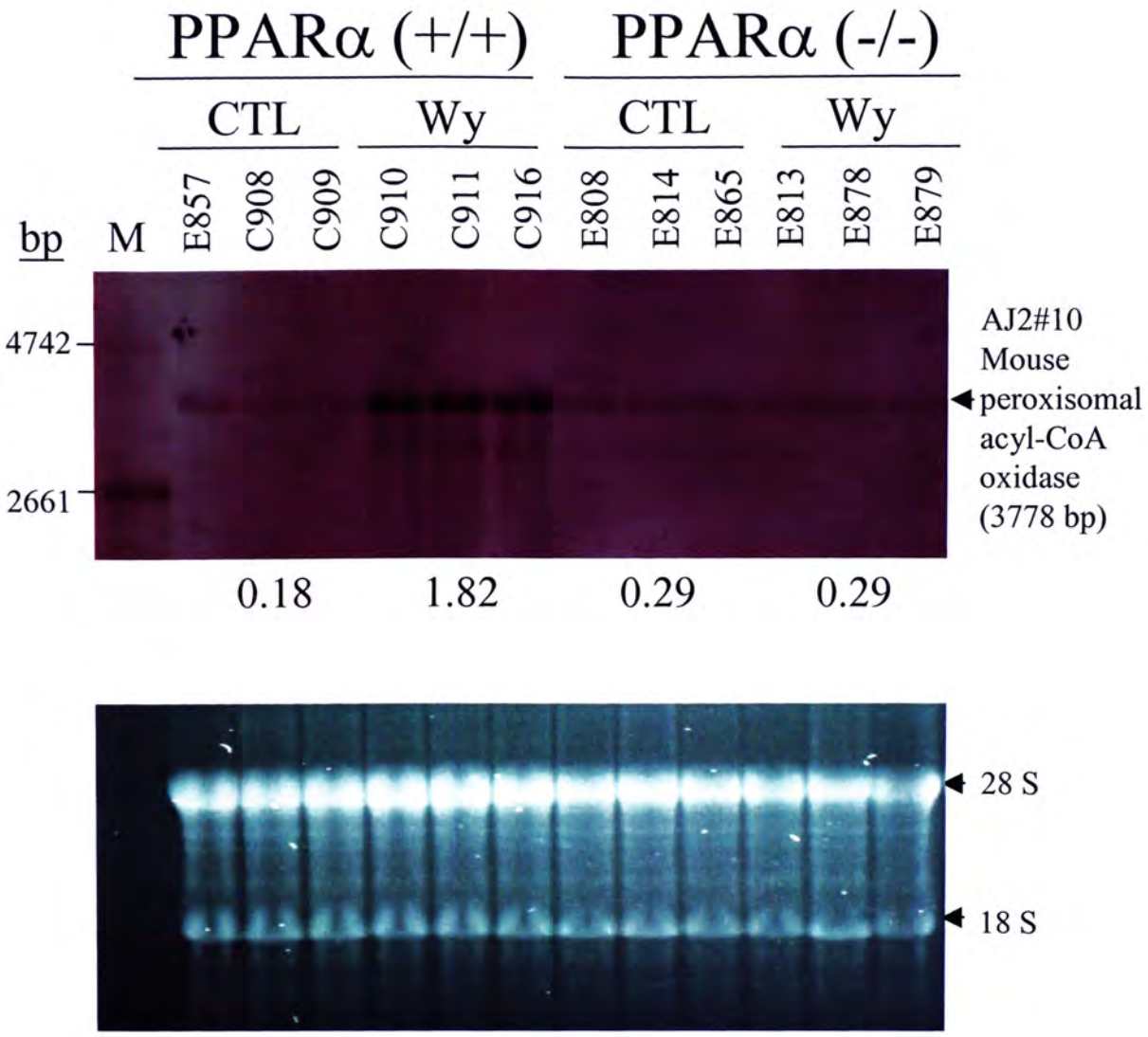

Figure 3.11.14. Confirmation of fluoroDD expression pattern of subclone AJ2#10 (peroxisomal acyl-CoA oxidase) by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

Table 3.11.4. Summary of Northern blot analysis of cDNA fragments subcloned from gels AF (AP10 and ARP13) and AJ (AP6 and ARP14)

Gel	Subclone I.D.	Differential display pattern				Gene homology (Transcript size)	Northern blot analysis densitometry reading (arbitary unit)					
		PPARα (+/+)		PPARα(-/-)			PPARα (+/+)			PPARα (-/-)		
		CTL	Wy	CTL	Wy		CTL	Wy	Fold change (Wy/CTL)	CTL	Wy	Fold change (Wy/CTL)
AF (AP10 & ARP13)	AF1#8	+	++	+	+	*Mus musculus* very-long-chain acyl-CoA synthetase, VLACS (2209 bp)	1.30 ± 0.21	2.70 ± 0.32	2.08	0.80 ± 0.17	1.10 ± 0.44	1.38
							++	+++		+	+	
	AF25#7	++	+	++	++	*Mus musculus* major urinary protein 2, MUP II (891 bp)	10.06 ± 1.04	0.84 ± 0.32	0.08	9.14 ± 1.57	10.61 ± 1.21	1.16
							+++	+		+++	+++	
AJ (AP6 & ARP14)	AJ1#5	++	+	++	++	Mouse carboxylesterase, Carboxylesterase (2006 bp)	1.82 ± 0.20	0.14 ± 0.03	0.08	2.51 ± 0.26	2.21 ± 0.97	0.88
							+++	-		+++	+++	
	AJ2#10	+	++	+	+	*Mus musculus* peroxisomal acyl-CoA oxidase, AOX (3778 bp)	0.18 ± 0.07	1.82 ± 0.10	10.11	0.29 ± 0.04	0.29 ± 0.02	1.00
							+	++		+	+	

Values represent mean ± S.D. '+' represents the presence of a band (cDNA) on fluoroDD gel or Northern blot, while '-' represents the corresponding band (cDNA) was not observed on the fluoroDD gel or Northern blot. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. The signal intensity of a band on Northern blot was quantified by MultiAnalyst.

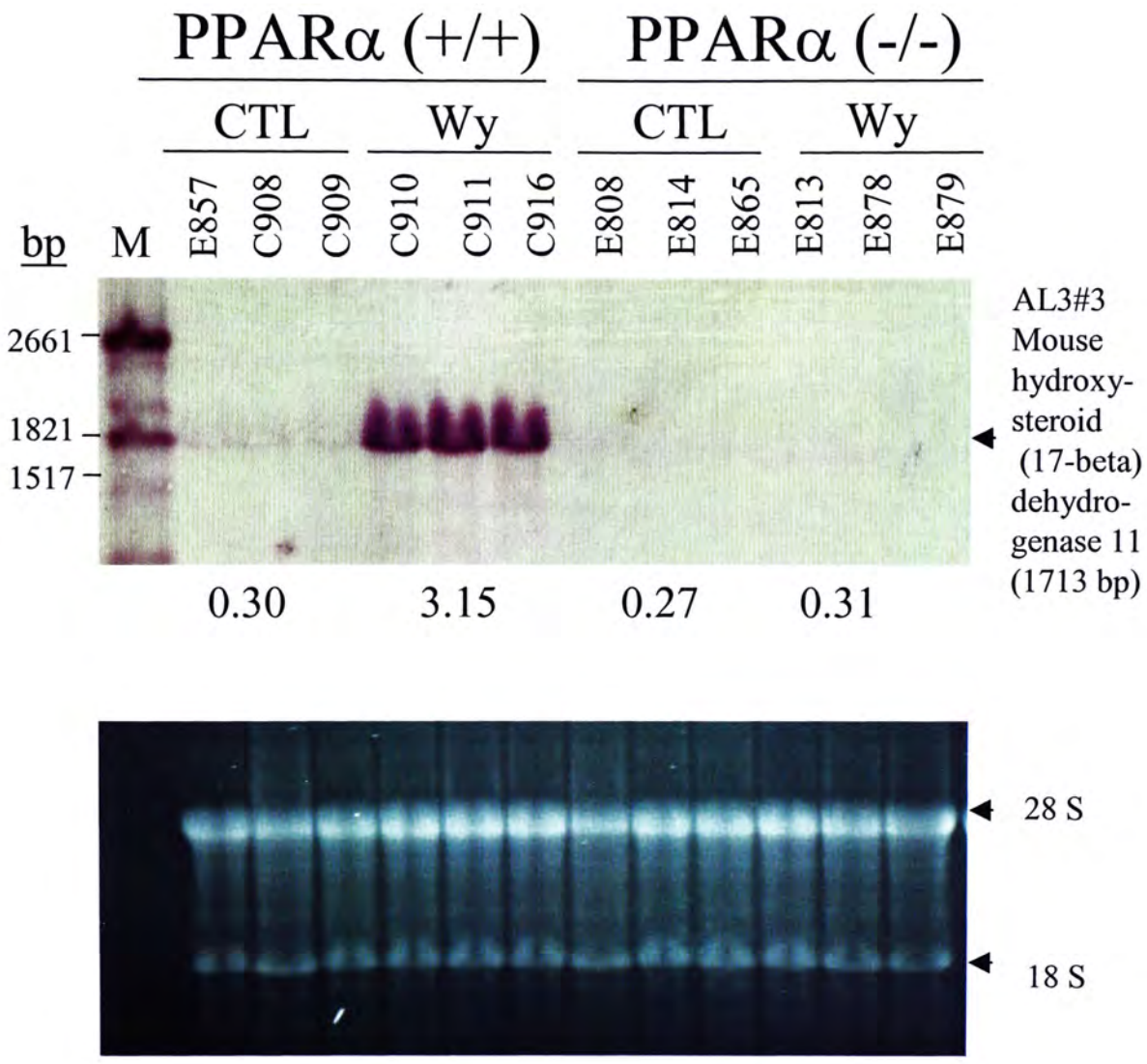

Figure 3.11.15. Confirmation of fluoroDD expression pattern of subclone AL3#3 [hydroxysteroid (17-beta) dehydrogenase 11] by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

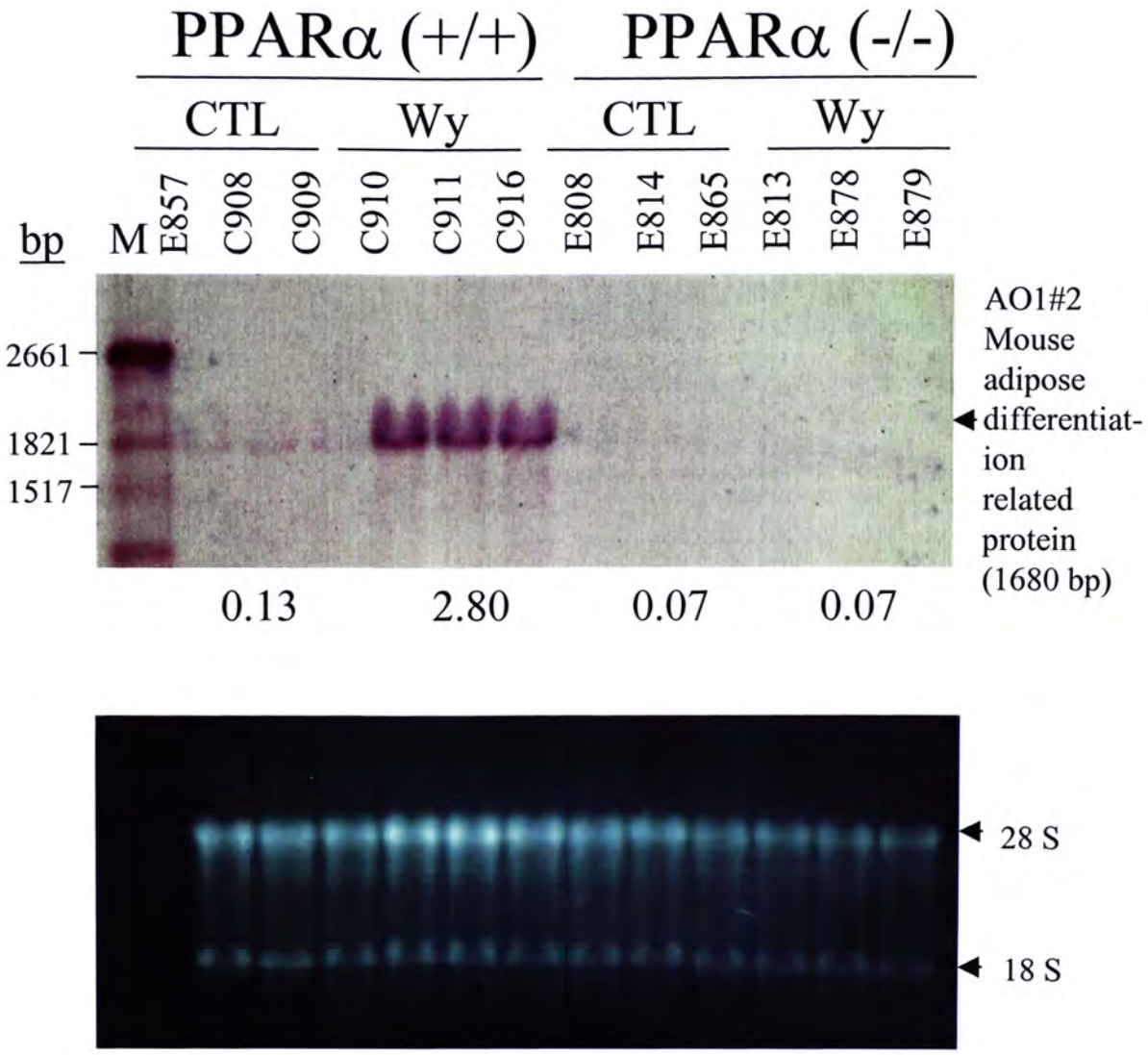

Figure 3.11.16. Confirmation of fluoroDD expression pattern of subclone AO1#2 (adipose differentiation related protein, ADFP) by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

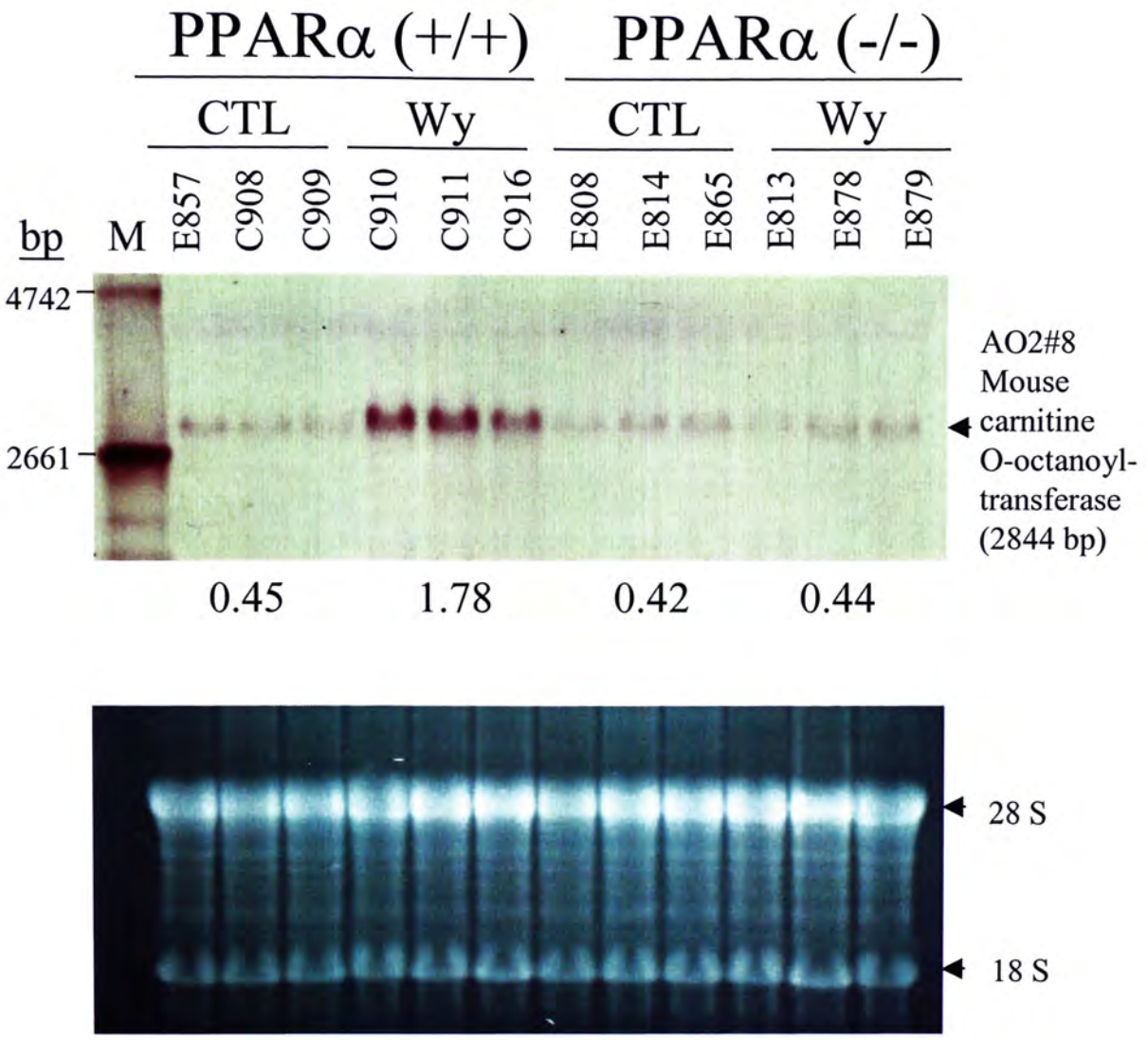

Figure 3.11.17. Confirmation of fluoroDD expression pattern of subclone AO2#8 (carnitine O-octanoyltransferase) by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

Table 3.11.5. Summary of Northern blot analysis of cDNA fragments subcloned from gels AL (AP7 and ARP15) and AO (AP5 and ARP10)

Gel	Subclone I.D.	Differential display pattern				Gene homology (Transcript size)	Northern blot analysis densitometry reading (arbitary unit)					
		PPARα (+/+)		PPARα(-/-)			PPARα (+/+)			PPARα (-/-)		
		CTL	Wy	CTL	Wy		CTL	Wy	Fold change (Wy/CTL)	CTL	Wy	Fold change (Wy/CTL)
AL (AP7 & ARP15)	AL3#3	+	++	+	+	*Mus musculus* hydroxysteroid (17-beta) dehydrogenase 11, HSD17β11 (1713 bp)	0.30 ± 0.07 +	3.15 ± 0.85 ++	10.50	0.27 ± 0.09 +	0.31 ± 0.07 +	1.15
AO (AP5 & ARP10)	AO1#2	+	++	+	+	Mouse adipose differentiation related protein, ADRP (1680 bp)	0.13 ± 0.06 -	2.08 ± 0.12 +	16.00	0.07 ± 0.05 -	0.07 ± 0.04 -	1.00
	AO2#8	+	++	+	+	*Mus musculus* carnitine O-octanoyltransferase, Crot (2844 bp)	0.45 ± 0.06 +	1.78 ± 0.32 ++	3.96	0.42 ± 0.12 +	0.44 ± 0.12 +	1.05

Values represent mean ± S.D. '+' represents the presence of a band (cDNA) on fluoroDD gel or Northern blot, while '-' represents the corresponding band (cDNA) was not observed on the fluoroDD gel or Northern blot. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. The signal intensity of a band on Northern blot was quantified by MultiAnalyst.

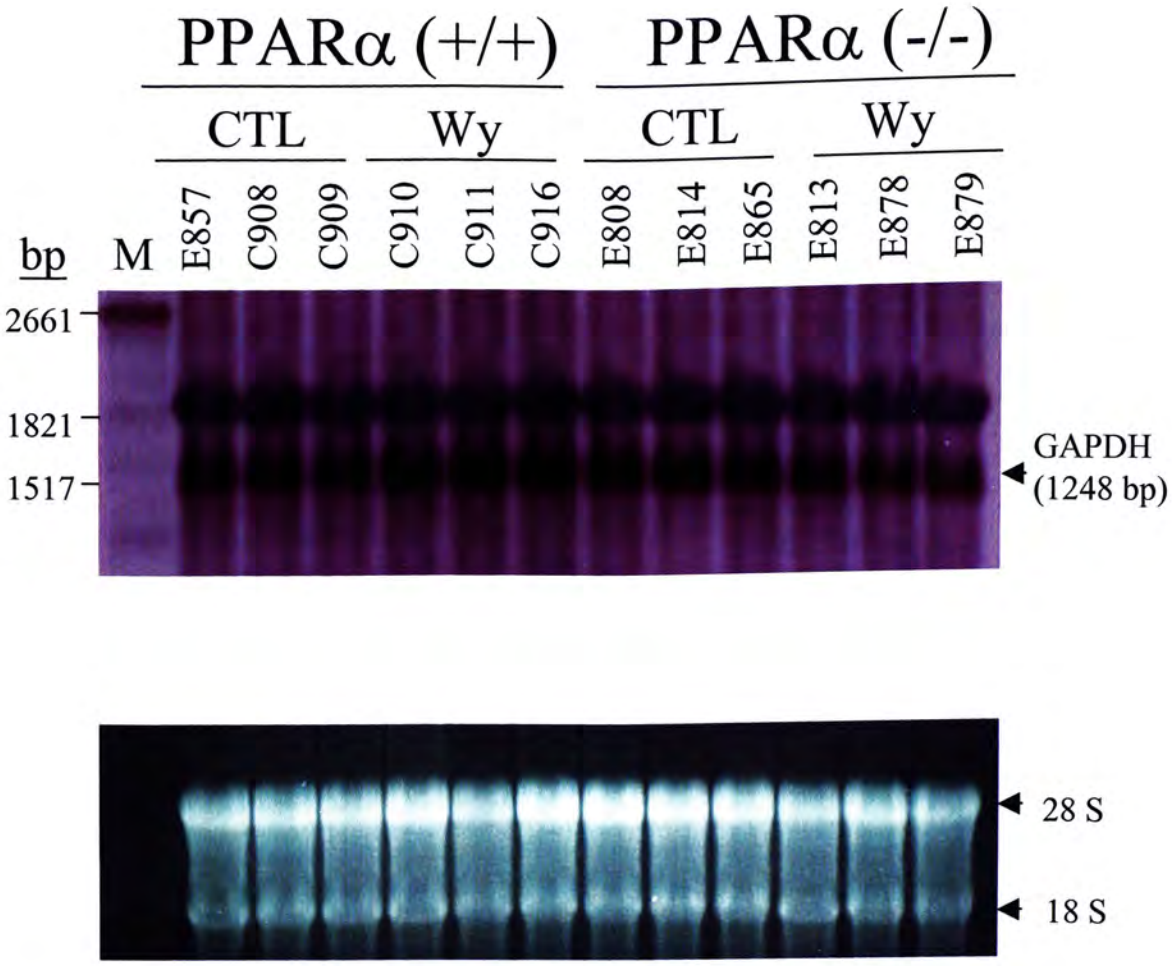

Figure 3.11.18. Up-regulation of GAPDH was observed in PPARα (+/+) mice fed with a 0.1% (w/w) Wy-14,643 diet for 11 months. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

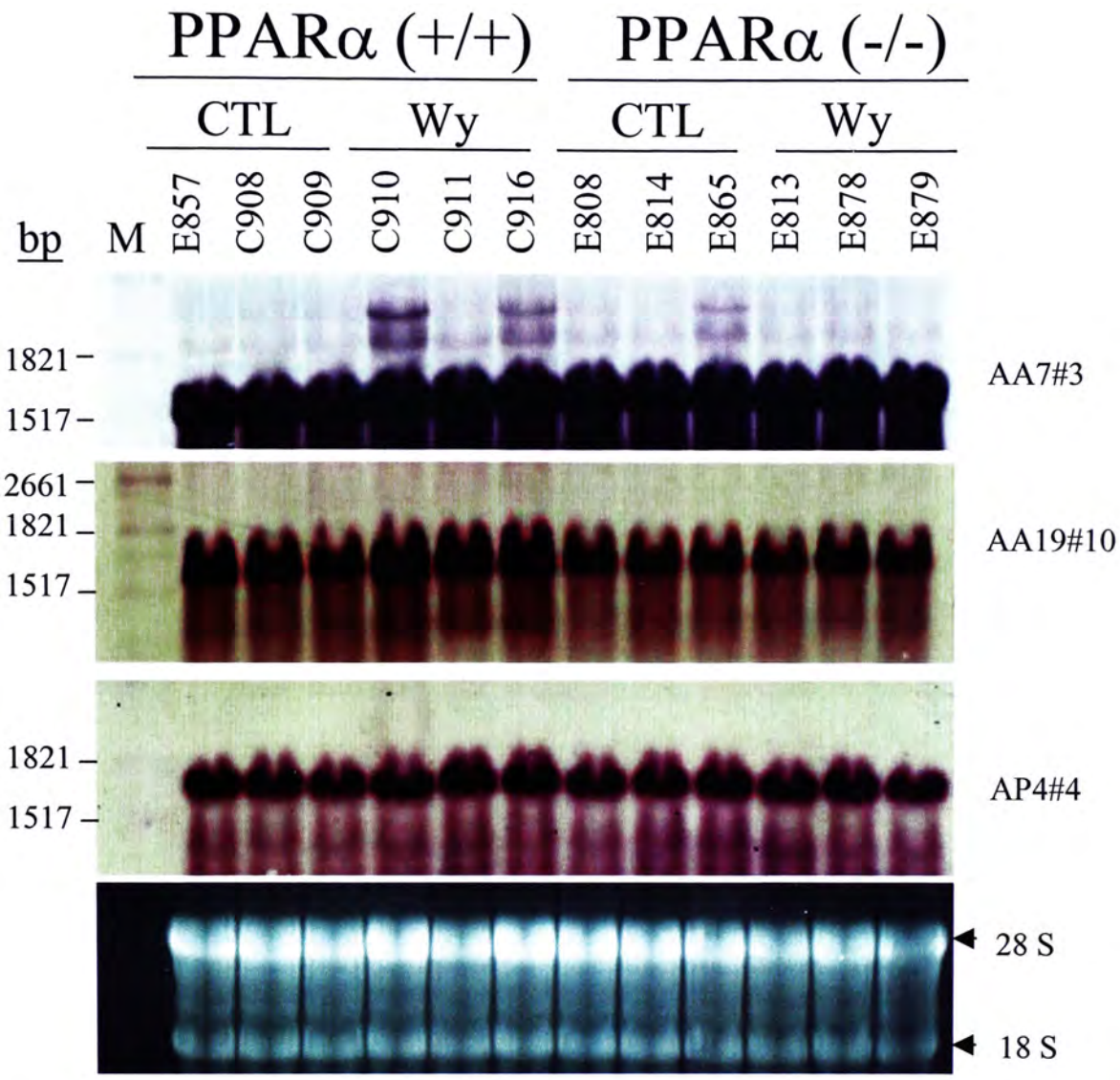

Figure 3.11.19. Confirmation of fluoroDD expression pattern of mitochondria by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

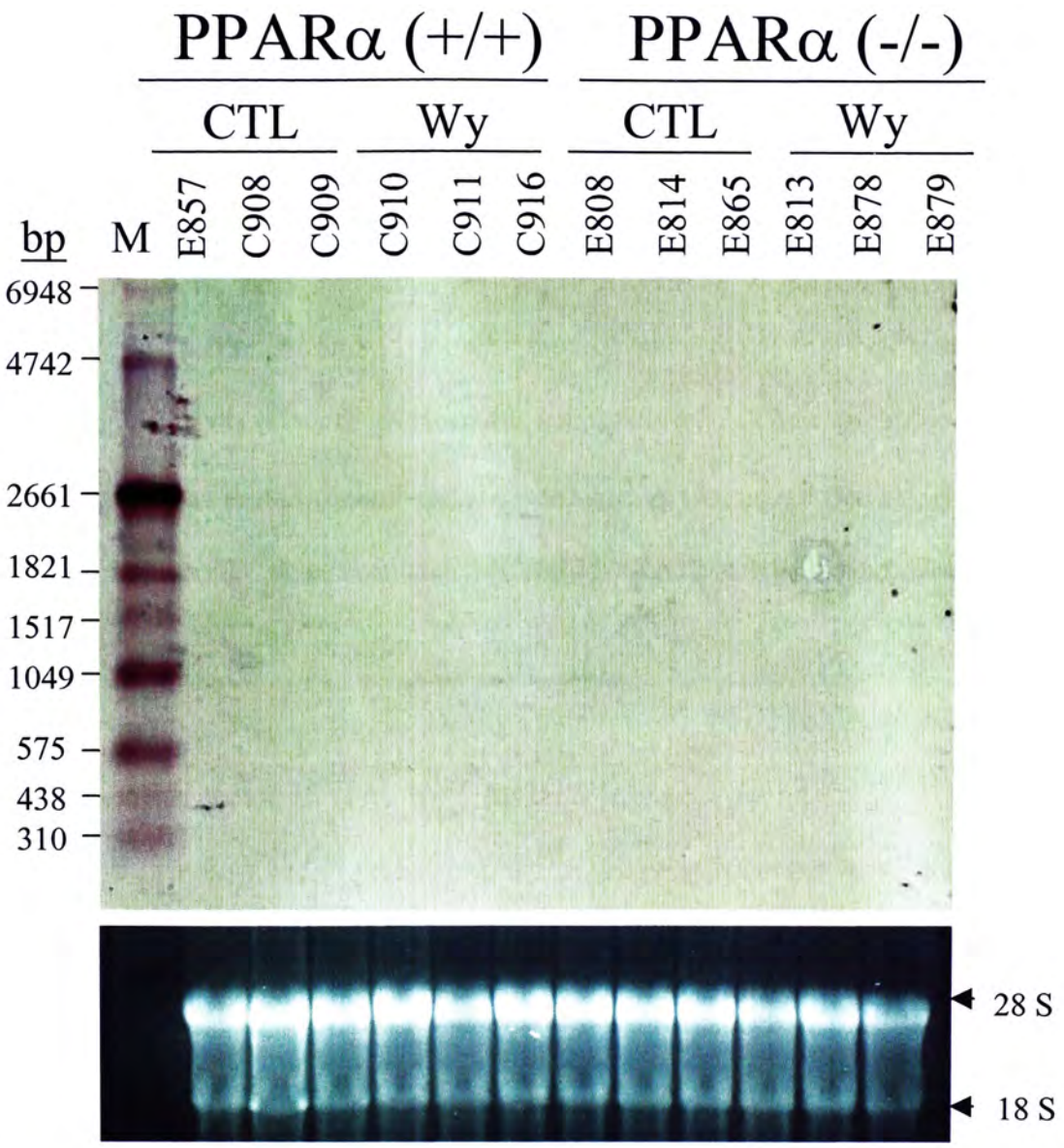

Figure 3.11.20. Confirmation of fluoroDD expression pattern of EST by Northern blot analysis. Total liver RNA (30 μg) of three mice each of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 11 months was separated on a 1% agarose-formaldehyde gel and transferred to a nylon membrane. The membrane was then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents. For normalization, ethidium bromide staining of RNA gel was used. M, RNA molecular weight marker I, DIG-labeled.

(UDP2b5)] gave different patterns from fluoroDD patterns in Northern blot analysis. It might be due to the co-migrated cDNA fragments in fluoroDD gel which have other fluoroDD pattern.

In order to study the time effect of the Wy-14,643 on the PPARα regulated gene, Northern blot analysis was performed with RNA samples from 24 hour, 1 week, 2 weeks, 6 months and 11 months feeding mice of all the four treatment groups. A total of sixteen were performed successfully. They included AA1#2 [mouse peroxisomal delta3, delta2-enoyl-Coenzyme A isomerase (Peci)]; AA10#1 [cysteine sulfinic acid decarboxylase (Csad)]; AA12#4 [mouse acetyl-Coenzyme A dehydrogenase, medium chain (MCAD)]; AB7#2 [mouse UDP-glucuronosyltransferase 2 family member 5, (UDP2b5)]; AB22#9 [peroxisome biogenesis factor 16 (Pex 16)]; AB25#6 [mouse cytochrome P450 4a14 (Cyp4a14)]; AC1#2 [mouse serine protease inhibitor, clade A, member 1a (SPI)]; AC2#2 [mouse peroxisomal bifunctional enzyme (PBFE)]; AD8#2 [mouse cytochrome P450 4a10 (Cyp4a10)]; AF1#8 [very-long-chain acyl-CoA synthetase (VLACS)]; AF25#7 [mouse major urinary protein II (MUP II)]; AJ1#5 [mouse carboxyesterase (carboxyesterase)]; AJ2#10 [mouse peroxisomal acyl-CoA oxidase (AOX)]; AL3#3 [mouse hydroxysteroid (17-beta) dehydrogenase 11, (Hsd17β11)]; AO1#2 [mouse adipose differentiation related protein (ADRP)] and AO2#8 [mouse carnitine O-octanoyltransferase (Crot)]. The signals were quantified by MultiAnalyst and fold change was calculated.

Up-regulation of AA1#2 [mouse peroxisomal delta3, delta2-enoyl-Coenzyme A isomerase (Peci) (Figure 3.11.21) (Table 3.11.6)] in PPARα (+/+) mice fed with the 0.1% Wy-14,643 diet was started at 24 hours gavage feeding and continued to 11 months feeding. Maximum induction was observed at 24 hours gavage feeding (6.6-

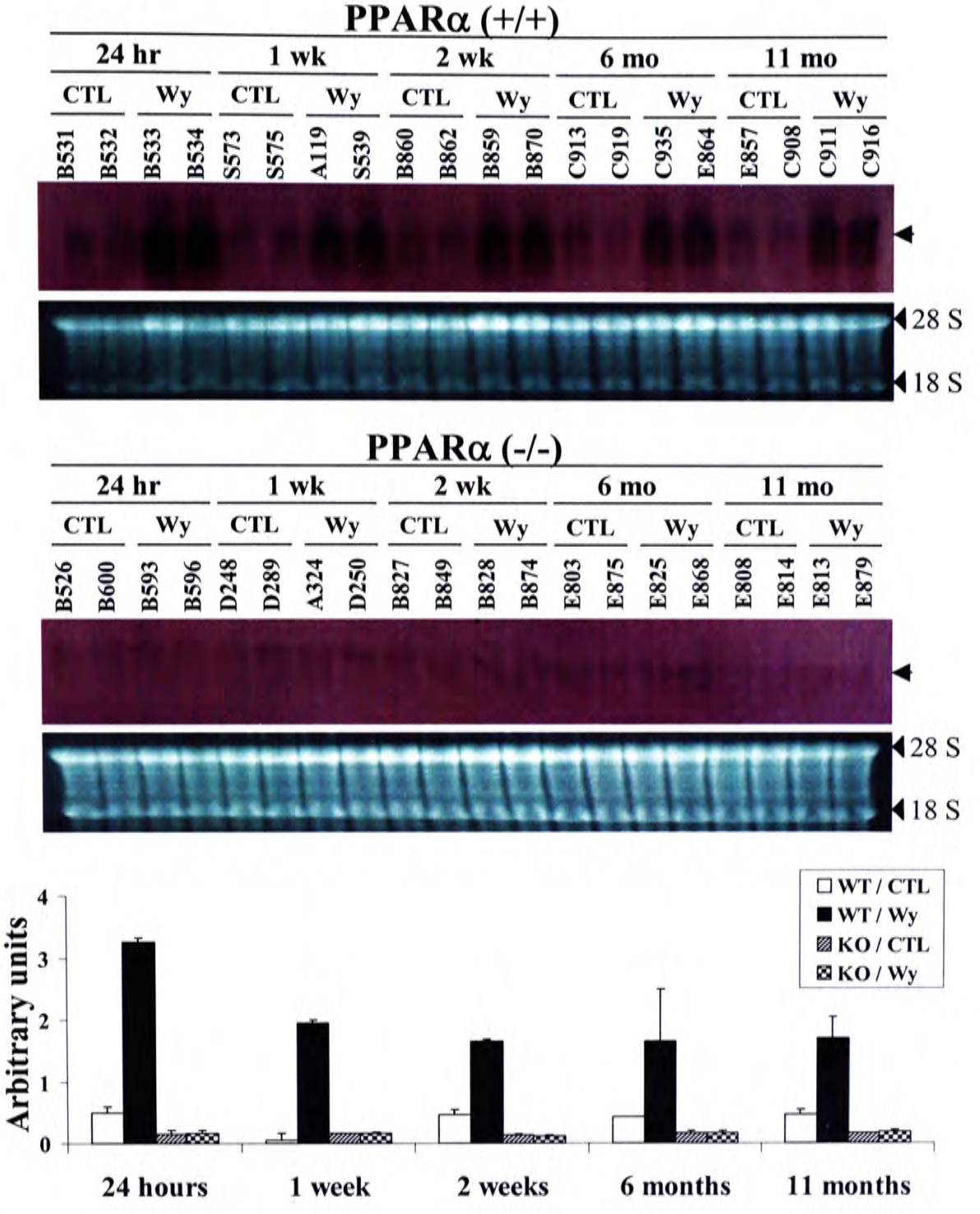

Figure 3.11.21. Temporal expression of AA1#2 (peroxisomal delta 3, delta 2-enoyl-coenzyme A isomerase). Total liver RNA (30 μg) of two mice of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week, 2 weeks, 6 months and 11 months treatment was separated on 1% agarose-formaldehyde gels and transferred to nylon membranes. The membranes were then hybridized with a PCR DIG-labeled cDNA probe and signals were detected by colorimetric method with NBT and BCIP reagents.

Table 3.11.6. Summary of Northern blot analysis on temporal expression patterns of cDNA fragments subcloned from gel AA (AP1 and ARP2)

Gel	Subclone I.D.	Gene homology (Transcript size)	Northern blot densitometry reading (arbitrary units)																			
			PPARα (+/+)										PPARα (-/-)									
			24 hr		1 wk		2 wk		6 mo		11 mo		24 hr		1 wk		2 wk		6 mo		11 mo	
			CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy
AA (AP1 & ARP2)	AA 1#2	*Mus musculus* peroxisomal delta 3, delta 2-enoyl-coenzyme A isomerase, Peci (1581 bp)	0.49 ± 0.11	3.25 ± 0.08	0.53 ± 0.11	1.95 ± 0.05	0.46 ± 0.09	1.69 ± 0.02	0.43 ± 0.00	1.66 ± 0.84	0.46 ± 0.08	1.70 ± 0.35	0.15 ± 0.06	0.17 ± 0.04	0.16 ± 0.01	0.16 ± 0.02	0.13 ± 0.02	0.12 ± 0.01	0.17 ± 0.03	0.16 ± 0.04	0.16 ± 0.01	0.18 ± 0.04
	Fold change (Wy/CTL)		6.63		3.67		3.67		3.86		3.70		1.13		2.00		0.92		0.94		1.13	
	AA10#1	*Mus musculus* cysteine sulfinic acid decarboxylase, Csad (2265 bp)	0.01 ± 0.02	0.02 ± 0.02	0.06 ± 0.02	0.12 ± 0.00	0.03 ± 0.00	0.10 ± 0.03	0.02 ± 0.01	0.09 ± 0.02	0.04 ± 0.02	0.11 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
	Fold change (Wy/CTL)		2.00		2.00		3.33		4.5		2.75		1.00		1.00		1.00		1.00		1.00	
	AA12#4	*Mus musculus* acetyl-coenzyme A dehydrogenase, medium chain, MCAD (1857 bp)	0.12 ± 0.07	0.46 ± 0.13	0.14 ± 0.03	0.57 ± 0.24	0.10 ± 0.08	0.32 ± 0.08	0.10 ± 0.03	0.27 ± 0.07	0.13 ± 0.01	0.41 ± 0.04	0.03 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
	Fold change (Wy/CTL)		3.83		4.07		3.20		2.70		3.15		1.00		1.00		1.00		1.00		1.00	

Values represent mean ± S.D. '+' represents the presence of a band (cDNA) on fluoroDD gel or Northern blot, while '-' represents the corresponding band (cDNA) was not observed on the fluoroDD gel or Northern blot. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. The signal intensity of a band on Northern blot was quantified by MultiAnalyst.

fold). However, no induction was observed in PPARα (-/-) mice fed with 0.1% Wy-14,643 when compared with the controls.

Very weak signal was obtained for the Northern blot analysis of time-dependent expression of fragment AA4#9 [mouse apolipoprotein A-V (Apoa5)].

Although weak signals were observed in the temporal expression of fragment AA10#1 [mouse cysteine sulfinic acid decarboxylase (Csad) (Figure 3.11.22) (Table 3.11.6)] consistent induction (2 to 5-fold) in 0.1% Wy-14,643 fed PPARα (+/+) was started at 1 week and continued to 11 months treatment. The PPARα (-/-) mice fed with the 0.1% Wy-14,643 diet did not show any induction when compared with the controls.

Similar to AA10#1, weak signals were found in the temporal expression of AA12#4 [mouse acetyl-Coenzyme A dehydrogenase, medium chain, MCAD (Figure 3.11.23) (Table 3.11.6)]. Consistent up-regulation (2 to 4-fold) started at 24 hours and continued to 11 months treatment of AA12#4 was observed. The up-regulation was not shown in 0.1% Wy-14,643-treated PPARα (-/-) after short-term and long-term treatment.

cDNA fragment AB7#2 [mouse UDP-glucuronosyltransferase 2 family member 5, UGT2b5 (Figure 3.11.24) (Table 3.11.7)] showed down-regulation which started at 2 weeks and continued to 11 months treatment. Maximum down-regulation (0.39-fold) was observed at 11 months treatment. However, down-regulation was not observed in the PPARα (-/-) mice fed with the 0.1% Wy-14,643 diet. Down-regulation bands were observed at about 2661 bp transcript in Wy-treated PPARα (+/+) mice. This might be the other member of the UDP-glucuronosyltransferase 2 family that has similar homology to UGT2b5, e.g. UGT2b4 which transcriptional size is 2093 bp and show high homology to UGT2b5.

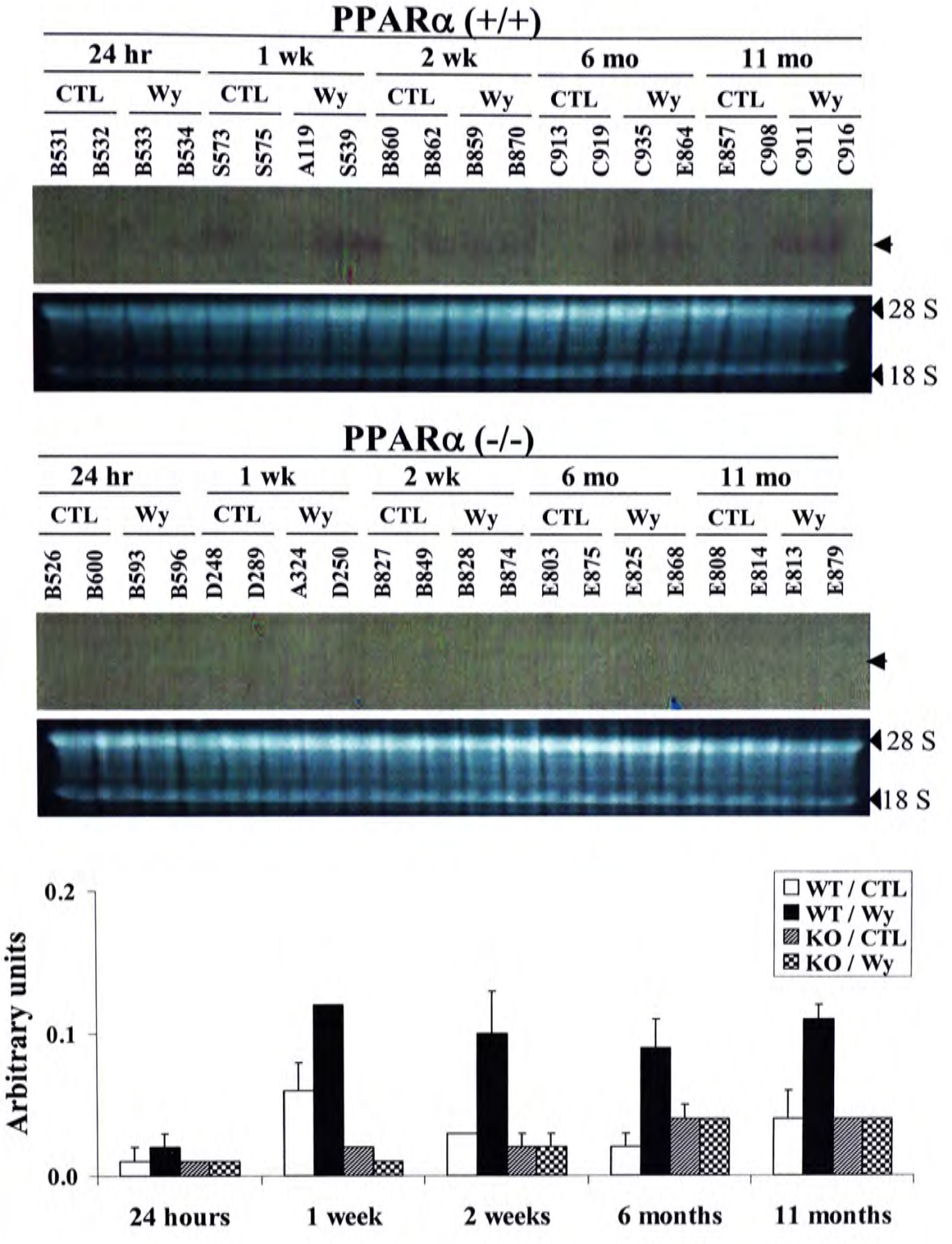

Figure 3.11.22. Temporal expression of AA10#1 (cysteine sulfinic acid decarboxylase). Total liver RNA (30 μg) of two mice of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week, 2 weeks, 6 months and 11 months treatment were separated on 1% agarose-formaldehyde gels and transferred to nylon membranes. The membranes were then hybridized with a PCR DIG cDNA probe and signals were detected by colorimetric method with NBT and BCIP reagents.

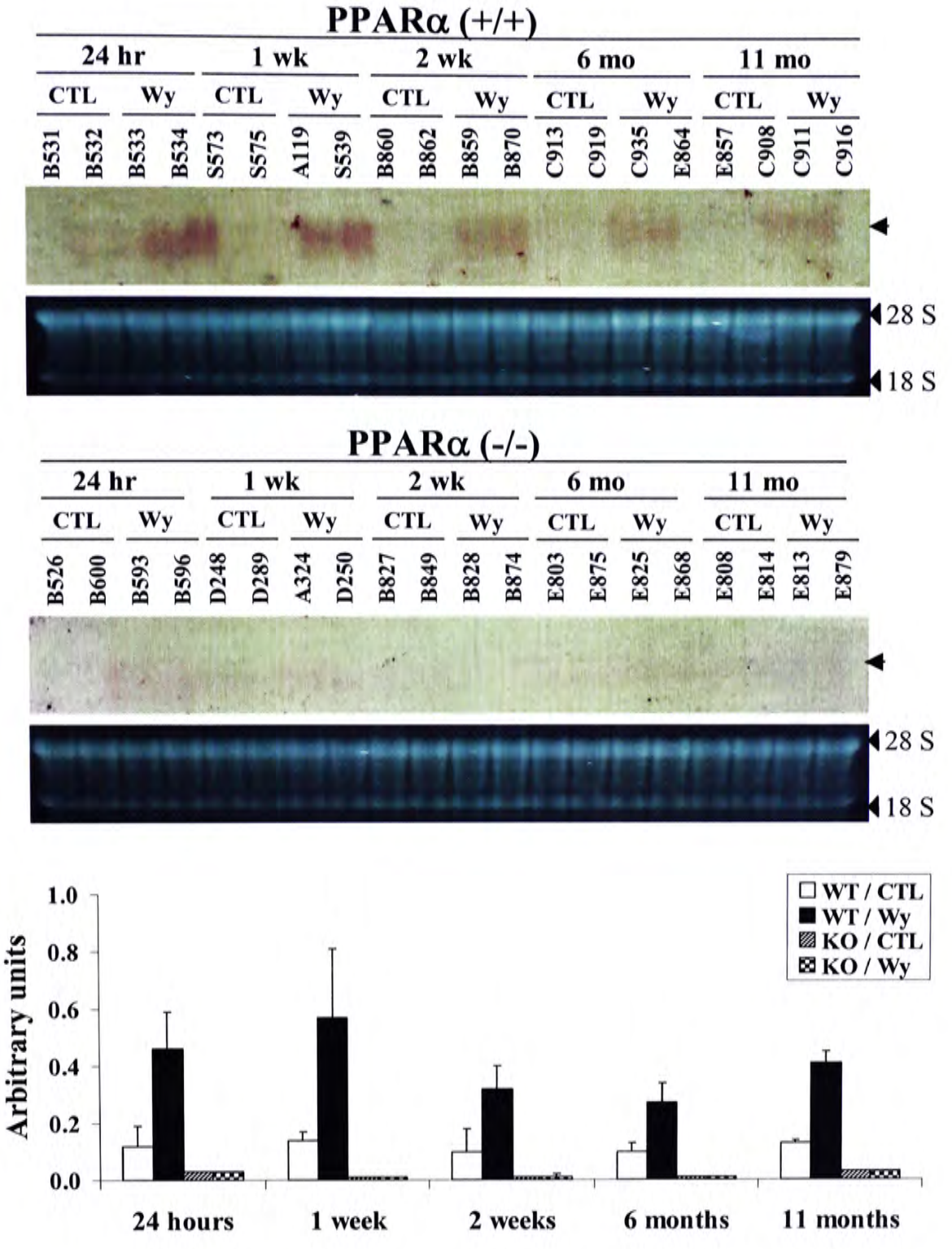

Figure 3.11.23. Temporal expression of AA12#4 (acetyl-Coenzyme A dehydrogenase, medium chain). Total liver RNA (30 μg) of two mice of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week, 2 weeks, 6 months and 11 months treatment were separated on 1% agarose-formaldehyde gels and transferred to nylon membranes. The membranes were then hybridized with a DIG-RNA labeled probe and signals were detected by colorimetric method with NBT and BCIP reagents.

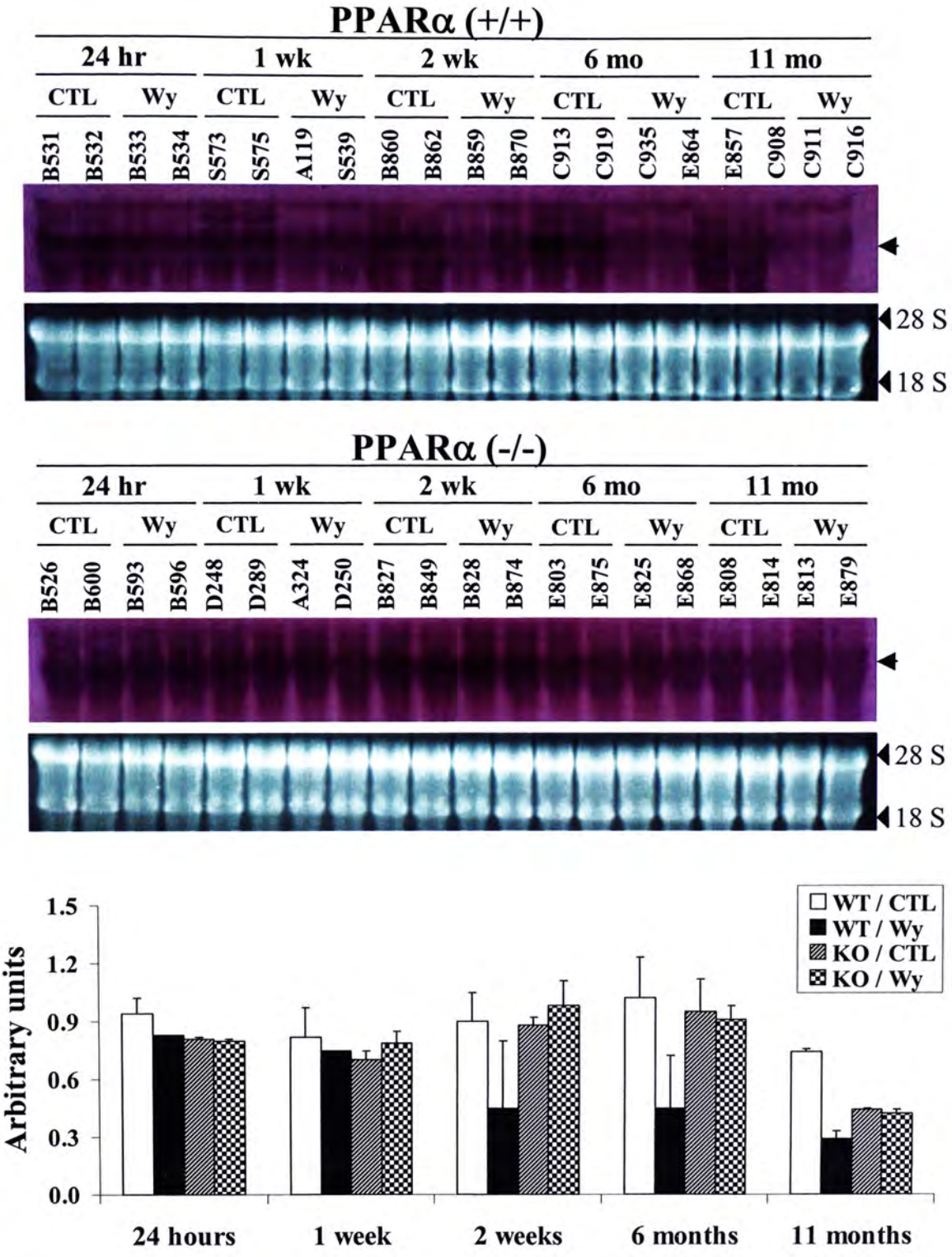

Figure 3.11.24. Temporal expression of AB7#2 (UDP-glucuronosyltransferase 2b5). Total liver RNA (30 μg) of two mice of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week, 2 weeks, 6 months and 11 months treatment were separated on 1% agarose-formaldehyde gels and transferred to nylon membranes. The membranes were then hybridized with a PCR DIG-labeled cDNA probe and signals were detected by colorimetric method with NBT and BCIP reagents.

Table 3.11.7. Summary of Northern blot analysis on temporal expression patterns of cDNA fragments subcloned from gel AB (AP3 and ARP3)

Gel	Subclone I.D.	Gene homology (Transcript size)	Northern blot densitometry reading (arbitrary units)																			
			PPARα (+/+)										PPARα (-/-)									
			24 hr		1 wk		2 wk		6 mo		11 mo		24 hr		1 wk		2 wk		6 mo		11 mo	
			CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy
AB (AP3 & ARP3)	AB7#2	Mouse UDP-glucuronosyl-transferase 2 family, member 5, UGT2b5 (1648 bp)	0.94 ± 0.08	0.83 ± 0.00	0.82 ± 0.15	0.75 ± 0.00	0.90 ± 0.15	0.45 ± 0.35	1.02 ± 0.21	0.45 ± 0.27	0.74 ± 0.02	0.29 ± 0.04	0.81 ± 0.01	0.80 ± 0.01	0.70 ± 0.05	0.79 ± 0.06	0.88 ± 0.04	0.98 ± 0.13	0.95 ± 0.17	0.91 ± 0.07	0.44 ± 0.01	0.42 ± 0.02
	Fold change (Wy/CTL)		0.88		0.91		0.50		0.44		0.39		0.99		1.13		1.11		0.96		0.95	
	AB22#9	*Mus musculus* peroxisome biogenesis factor 16, Pex16 (1221 bp)	0.10 ± 0.02	0.33 ± 0.19	0.16 ± 0.01	0.49 ± 0.04	0.15 ± 0.03	0.42 ± 0.04	0.15 ± 0.03	0.45 ± 0.16	0.11 ± 0.01	0.37 ± 0.04	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.05 ± 0.1	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
	Fold change (Wy/CTL)		3.30		3.06		2.80		3.00		3.36		1.00		1.00		1.00		1.00		1.00	
	AB25#6	*Mus musculus* Cyp4a14 (2547 bp)	0.02 ± 0.00	1.03 ± 0.08	0.03 ± 0.03	2.03 ± 0.56	0.02 ± 0.01	1.23 ± 0.26	0.03 ± 0.01	1.27 ± 0.09	0.03 ± 0.01	0.91 ± 0.14	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.03	0.02 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.05 ± 0.01	0.05 ± 0.01
	Fold change (Wy/CTL)		51.50		67.67		61.50		42.33		30.33		1.50		1.00		1.00		1.00		1.00	

Values represent mean ± S.D. '+' represents the presence of a band (cDNA) on fluoroDD gel or Northern blot, while '-' represents the corresponding band (cDNA) was not observed on the fluoroDD gel or Northern blot. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. The signal intensity of a band on Northern blot was quantified by MultiAnalyst.

Subcloned cDNA fragment AB22#9 [mouse peroxisome biogenesis factor 16 (Pex 16) (Figure 3.11.25) (Table 3.11.7)] showed consistent (2 to 3-fold) induction which started at 24 hours and continued to 11 months treatment. In PPARα (-/-) mice fed with the 0.1% Wy-14,643 diet, no induction was observed after both short-term and long-term treatment.

Very obvious up-regulation of AB25#6 [mouse cytochrome P450 4a14 (Cyp4a14) (Figure 3.11.26) (Table 3.11.7)] started at 24 hours and induced consistently until 11 months treatment was observed. The induction was not observed in PPARα (-/-) mice fed with the 0.1% Wy-14,643.

cDNA fragment AC1#2 [mouse serine protease inhibitor, clade A, member 1a, (SPI) (Figure 3.11.27) (Table 3.11.8)] showed down-regulation in the PPARα (+/+) mice fed with the 0.1% Wy-14,643 diet. The down-regulation was started at 1week and persisted to 11 months treatment. Maximum down-regulation was observed at 11 months treatment (0.17-fold). Down-regulation was not observed in the PPARα (-/-) mice fed with the 0.1% Wy-14,643 diet.

Subcloned cDNA fragment AC2#2 [mouse peroxisomal bifunctional enzyme (PBFE) (Figure 3.11.28) (Table 3.11.8)] was consistently up-regulated at 24 hours, 1 week, 2 weeks, 6 months and 11 months in the 0.1% Wy-14,643 fed PPARα (+/+) mice. In PPARα (-/-) mice fed with the 0.1% Wy-14,643 diet, no induction was observed after both short-term and long-term treatment.

cDNA fragment AD8#2 [mouse cytochrome P450 4a10 (Cyp4a10) (Figure 3.11.29) (Table 3.11.8)] was induced in 0.1% Wy-14,643 fed PPARα (+/+) mice. The induction was observed since 24 hours. Maximum induction was observed at 1 week (33-fold) and up-regulation was continued to 11 months (13-fold). No induction was observed in the PPARα (-/-) mice fed with the 0.1% Wy-14,643 diet.

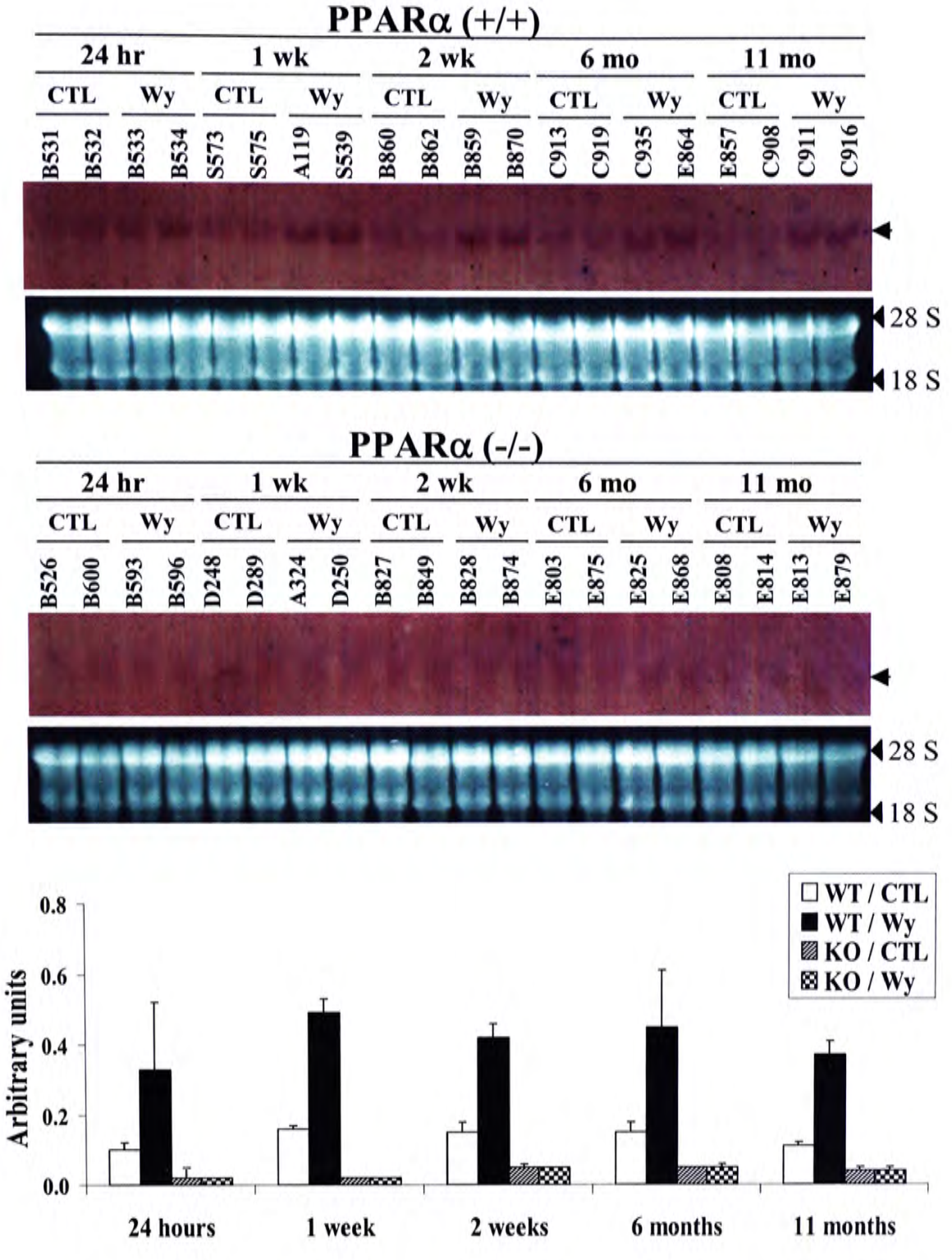

Figure 3.11.25. Temporal expression of AB22#9 (peroxisome biogenesis factor 16). Total liver RNA (30 μg) of two mice of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week, 2 weeks, 6 months and 11 months treatment were separated on 1% agarose-formaldehyde gels and transferred to nylon membranes. The membranes were then hybridized with a PCR DIG-labeled cDNA probe and signals were detected by colorimetric method with NBT and BCIP reagents.

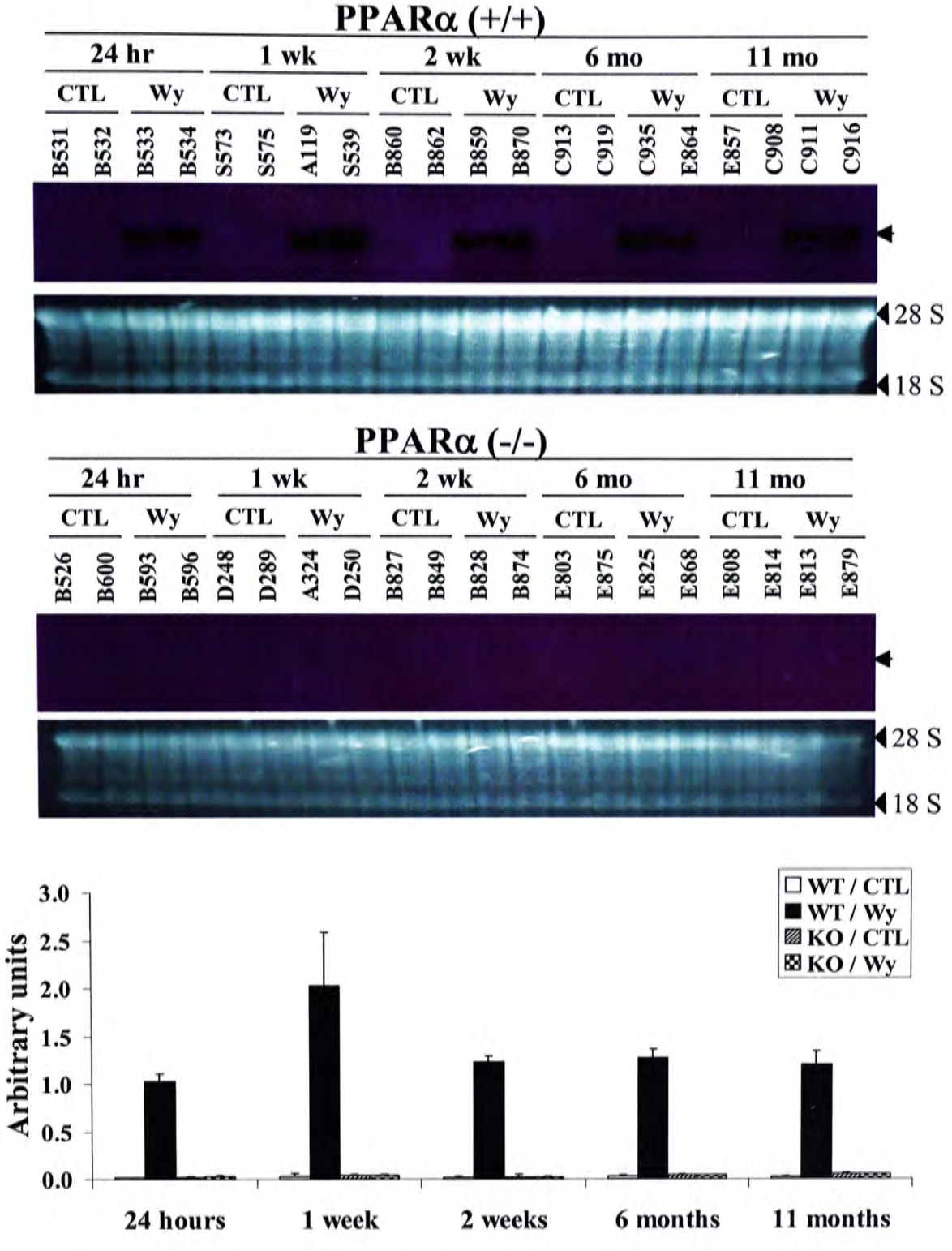

Figure 3.11.26. Temporal expression of AB25#6 (cytochrome P450, family 4, subfamily a, polypeptide 14). Total liver RNA (30 μg) of two mice of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week, 2 weeks, 6 months and 11 months treatment were separated on 1% agarose-formaldehyde gels and transferred to nylon membranes. The membranes were then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents.

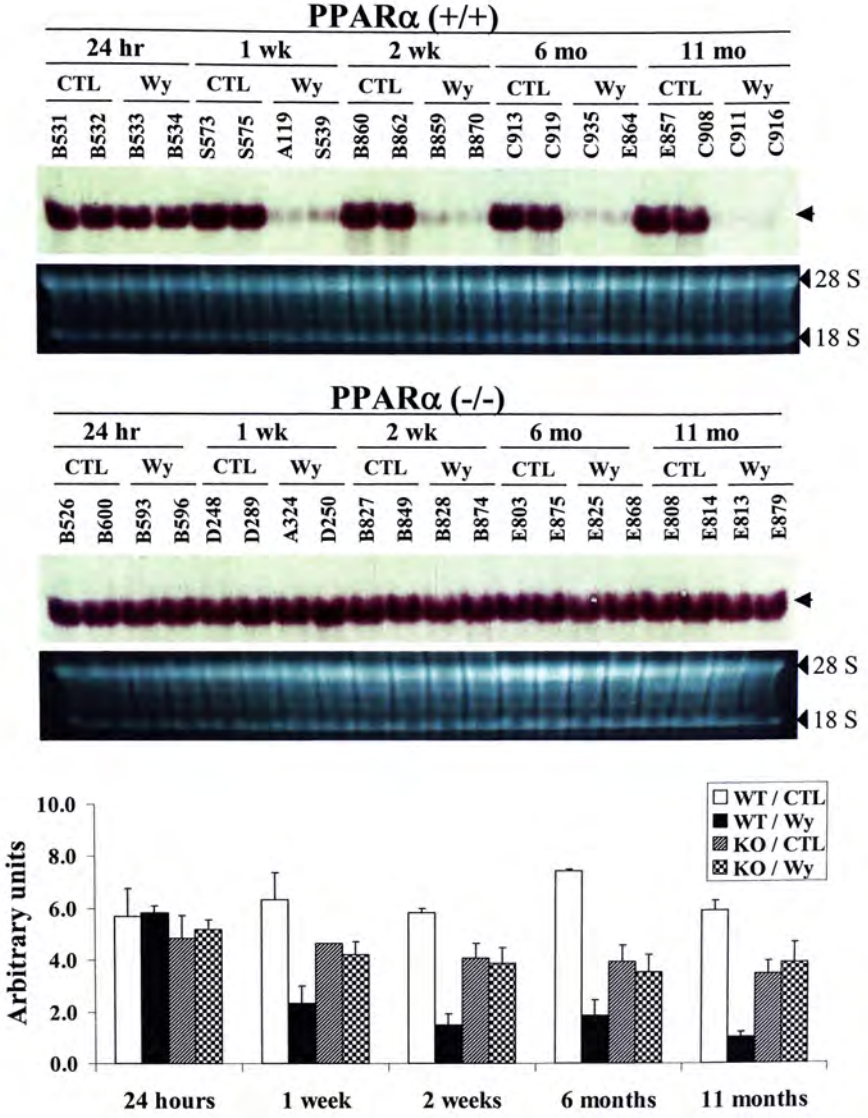

Figure 3.11.27. Temporal expression of AC1#2 (serine protease inhibitor). Total liver RNA (30 μg) of two mice of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week, 2 weeks, 6 months and 11 months treatment were separated on 1% agarose-formaldehyde gels and transferred to nylon membranes. The membranes were then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents.

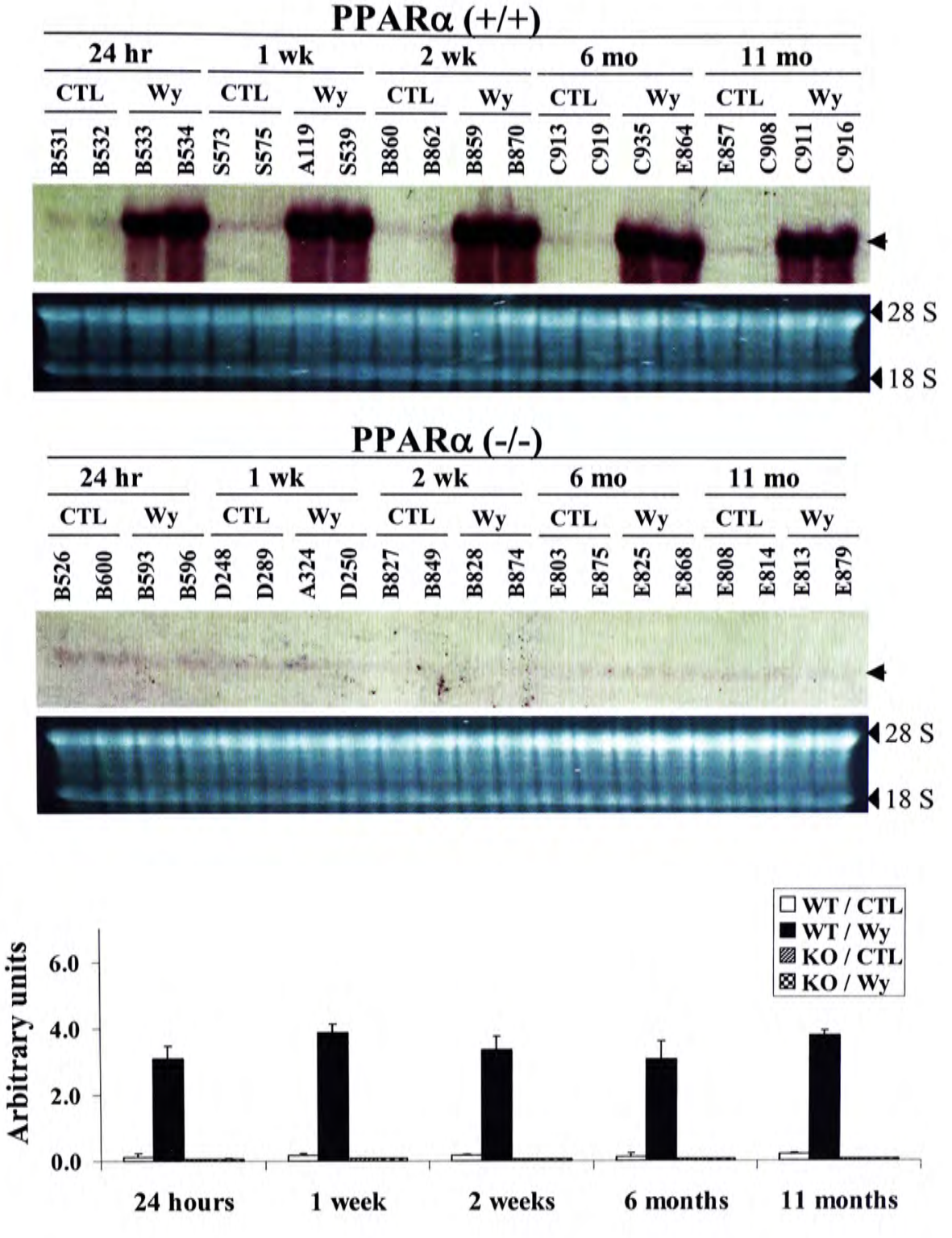

Figure 3.11.28. Temporal expression of AC2#2 (peroxisomal bifunctional enzyme). Total liver RNA (30 μg) of two mice of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week, 2 weeks, 6 months and 11 months treatment were separated on 1% agarose-formaldehyde gels and transferred to nylon membranes. The membranes were then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents.

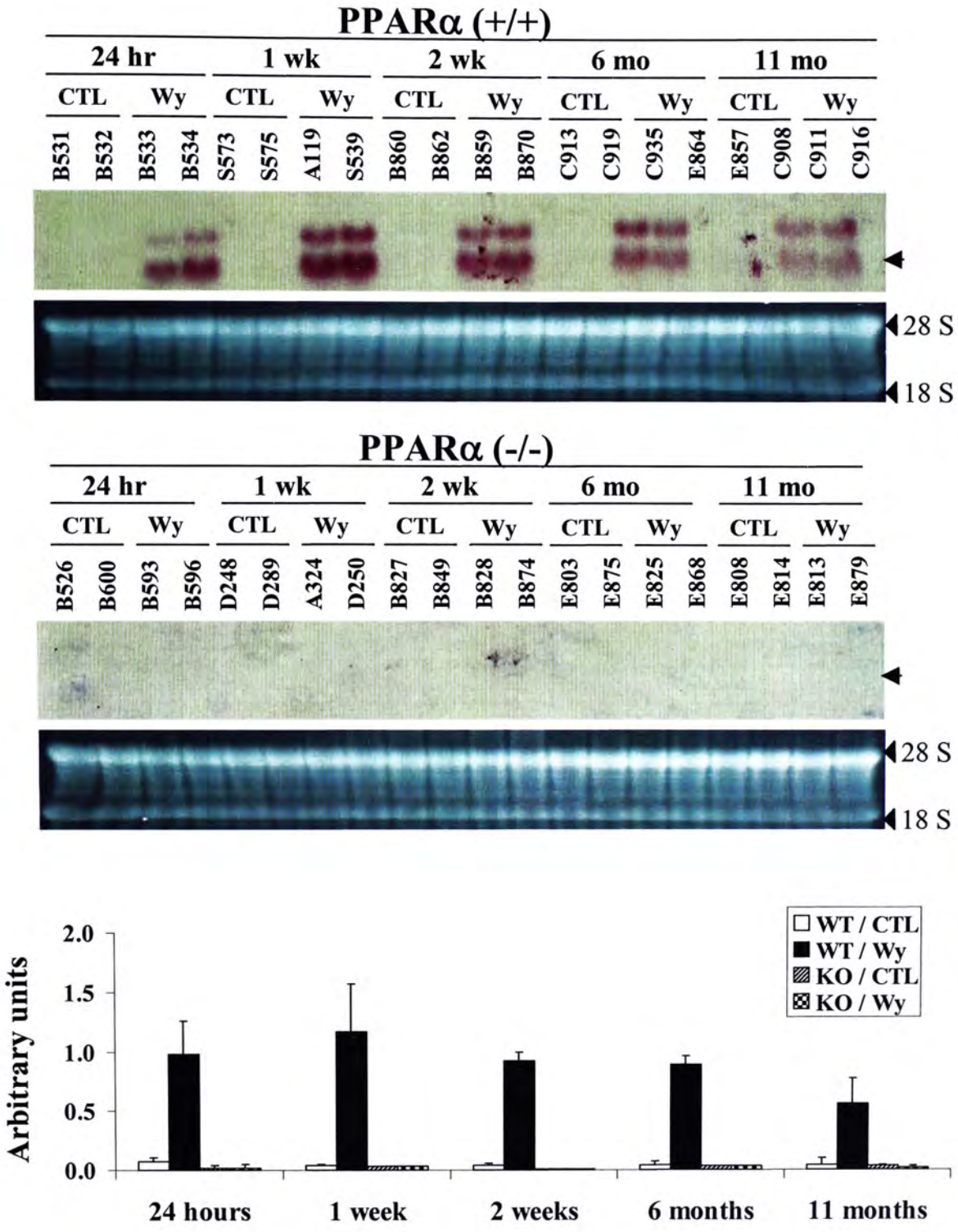

Figure 3.11.29. Temporal expression of AD8#2 (cytochrome P450, family 4, subfamily a, polypeptide 10). Total liver RNA (30 μg) of two mice of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week, 2 weeks, 6 months and 11 months treatment were separated on 1% agarose-formaldehyde gels and transferred to nylon membranes. The membranes were then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents.

Table 3.11.8. Summary of Northern blot analysis on temporal expression patterns of cDNA fragments subcloned from gels AC (AP2 and ARP19) and AD (AP2 and ARP18)

Gel	Subclone I.D.	Gene homology (Transcript size)	Northern blot densitometry reading (arbitrary units)																			
			PPARα (+/+)										PPARα (-/-)									
			24 hr		1 wk		2 wk		6 mo		11 mo		24 hr		1 wk		2 wk		6 mo		11 mo	
			CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy
AC (AP2 & ARP19)	AC1#2	*Mus musculus* serine (or cysteine) proteinase inhibitor, SPI (1393 bp)	5.68 ± 1.08	5.80 ± 0.29	6.32 ± 1.05	2.33 ± 0.66	5.83 ± 0.16	1.47 ± 0.45	7.43 ± 0.09	1.84 ± 0.61	5.91 ± 0.40	1.01 ± 0.20	4.86 ± 0.86	5.19 ± 0.37	4.63 ± 0.01	4.22 ± 0.49	4.07 ± 0.59	3.88 ± 0.59	3.95 ± 0.64	3.53 ± 0.68	3.46 ± 0.50	3.90 ± 0.79
	Fold change (Wy/CTL)		1.02		0.37		0.25		0.25		0.17		1.07		0.91		0.95		0.89		1.13	
	AC2#2	*Mus musculus* peroxisomal bifunctional enzyme (PBFE) (2979 bp)	0.11 ± 0.11	3.09 ± 0.39	0.18 ± 0.25	3.89 ± 0.25	0.18 ± 0.02	3.36 ± 0.40	0.13 ± 0.09	3.06 ± 0.57	0.21 ± 0.03	3.80 ± 0.12	0.06 ± 0.01	0.06 ± 0.02	0.08 ± 0.01	0.08 ± 0.00	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.00	0.06 ± 0.01	0.05 ± 0.02	0.05 ± 0.01
	Fold change (Wy/CTL)		30.90		21.61		18.67		23.54		18.10		1.00		1.00		1.00		1.00		1.00	
AD (AP2 & ARP18)	AD8#2	*Mus musculus* Cyp4a10 (2085 bp)	0.07 ± 0.04	0.98 ± 0.28	0.04 ± 0.01	1.17 ± 0.40	0.04 ± 0.02	0.92 ± 0.08	0.04 ± 0.03	0.89 ± 0.07	0.04 ± 0.06	0.56 ± 0.21	0.02 ± 0.02	0.02 ± 0.03	0.03 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.02 ± 0.01
	Fold change (Wy/CTL)		14.00		20.25		23.00		22.25		14.00		1.00		1.00		1.00		1.00		0.67	

Values represent mean ± S.D. '+' represents the presence of a band (cDNA) on fluoroDD gel or Northern blot, while '-' represents the corresponding band (cDNA) was not observed on the fluoroDD gel or Northern blot. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. The signal intensity of a band on Northern blot was quantified by MultiAnalyst.

cDNA fragment AF1#8 [mouse very-long-chain acyl-CoA synthetase (VLACS) (Figure 3.11.30) (Table 3.11.9)] was up-regulated consistently (about 2-fold which was started at 24 hours and continued to 11 months. The up-regulation was not shown in PPARα (-/-) mice fed with the 0.1% Wy-14,643 diet.

cDNA fragment AF25#7 [mouse major urinary protein II (MUP II) (Figure 3.11.31) (Table 3.11.9)] was down-regulated in PPARα (+/+) mice fed with the 0.1% Wy-14,643 diet. This down-regulation was started at 2 weeks and maximum down-regulation was found at 11 months treatment (0.01-fold). No down-regulation was observed in the PPARα (-/-) mice fed with the 0.1% Wy-14,643 diet after short-term and long-term treatment.

Down-regulation of AJ1#5 [mouse carboxyesterase (carboxyestersae) (Figure 3.11.32) (Table 3.11.10)] started at 1 week and maximum down-regulation (0.05-fold) was found at 11 months 0.1% Wy-14,643 treatment. No down-regulation was observed in the PPARα (-/-) mice fed with the 0.1% Wy-14,643 diet after short-term and long-term treatment.

cDNA fragment AJ2#10 [mouse peroxisomal acyl-CoA oxidase (AOX) (Figure 3.11.33) (Table 3.11.10)] was up-regulated in 0.1% Wy-14,643 treated PPARα (+/+) mice when compared with the controls. Maximum induction was observed at 24 hours and 1 week 0.1% Wy-14,643 treatment of PPARα (+/+) mice. The induction was persisted to 11 months and not found in PPARα (-/-) mice fed with the 0.1% Wy-14,643 diet.

Up-regulation of AL3#3 [mouse hydroxysteroid (17-beta) dehydrogenase 11 (HSD17β11) (Figure 3.11.34) (Table 3.11.10)] was found in 0.1% Wy-14,643 treated PPARα (+/+) mice. Induction of AL3#3 was started at 24 hours and continued to 11

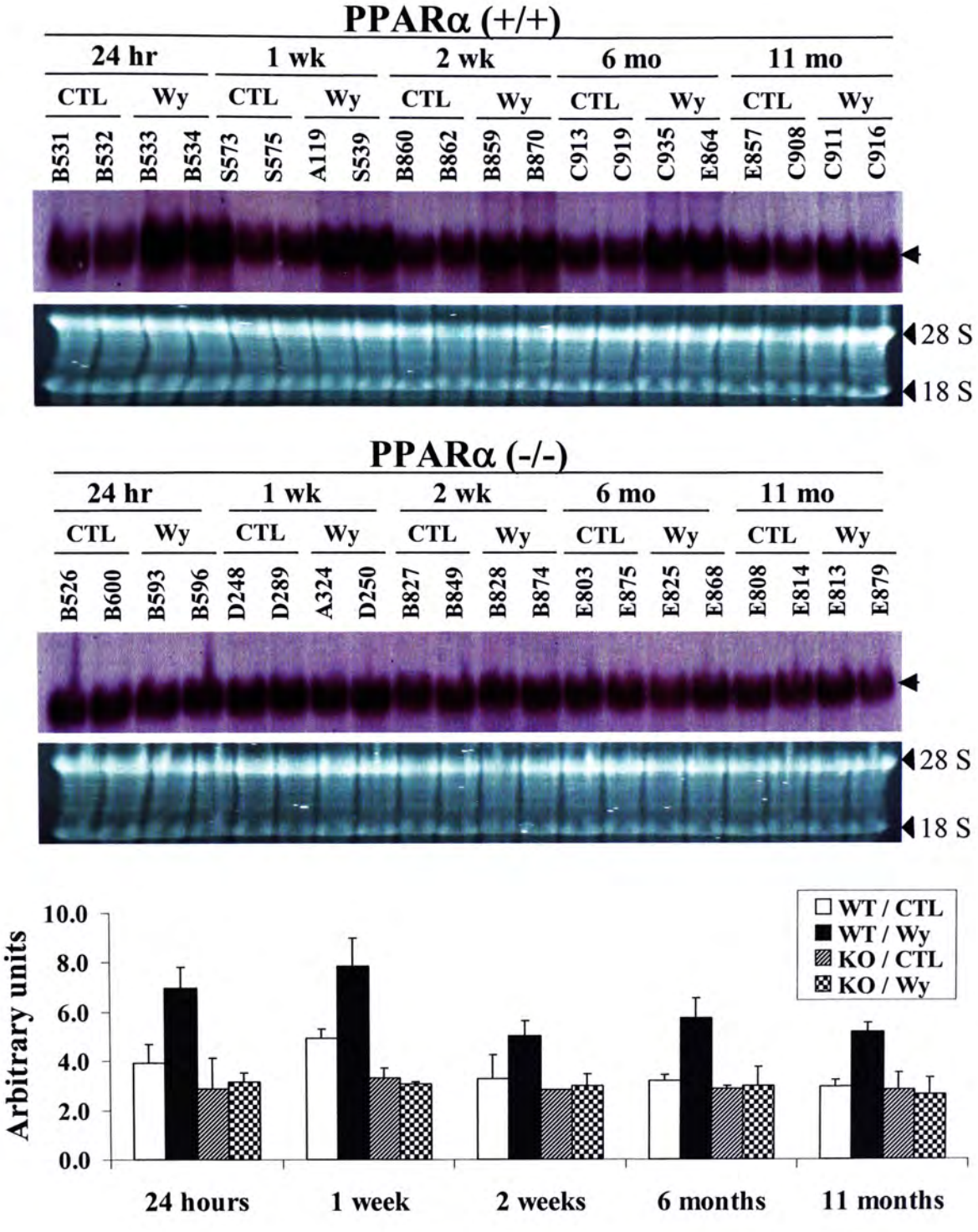

Figure 3.11.30. Temporal expression of AF1#8 (very long chain acyl-CoA synthetase). Total liver RNA (30 μg) of two mice of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week, 2 weeks, 6 months and 11 months treatment were separated on 1% agarose-formaldehyde gels and transferred to nylon membranes. The membranes were then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents.

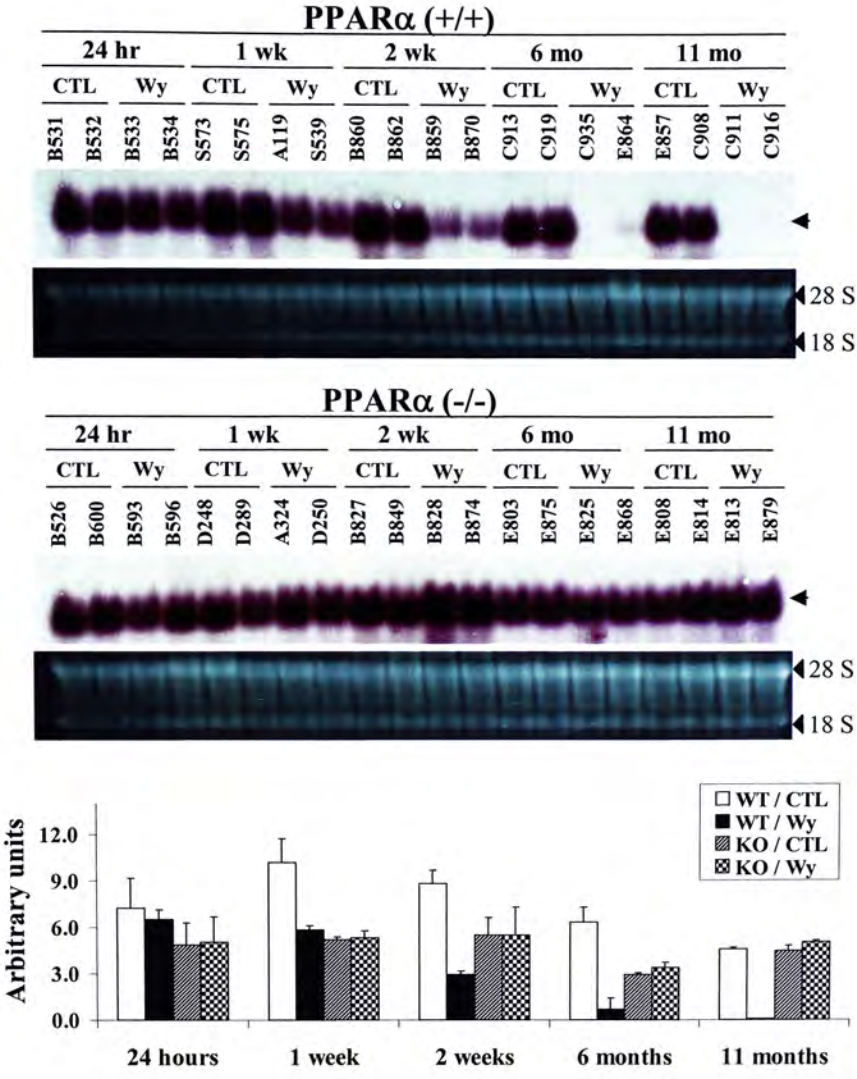

Figure 3.11.31. Temporal expression of AF25#7 (mouse major urinary protein II). Total liver RNA (30 μg) of two mice of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week, 2 weeks, 6 months and 11 months treatment were separated on 1% agarose-formaldehyde gels and transferred to nylon membranes. The membranes were then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents.

Table 3.11.9. Summary of Northern blot analysis on temporal expression patterns of cDNA fragments subcloned from gel AF (AP10 and ARP 13)

Gel	Subclone I.D.	Gene homology (Transcript size)	Northern blot densitometry reading (arbitrary units)																			
			PPARα (+/+)										PPARα (-/-)									
			24 hr		1 wk		2 wk		6 mo		11 mo		24 hr		1 wk		2 wk		6 mo		11 mo	
			CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy
AF (AP10 & ARP13)	AF1#8	*Mus musculus* very-long-chain acyl-CoA synthetase, VLACS (2209 bp)	3.93 ± 0.77	6.98 ± 0.84	4.94 ± 0.37	7.85 ± 1.15	3.29 ± 0.95	5.01 ± 0.61	3.21 ± 0.25	5.73 ± 0.84	2.91 ± 0.25	5.17 ± 0.38	2.89 ± 1.22	3.17 ± 0.37	3.34 ± 0.40	3.06 ± 0.11	2.83 ± 0.02	2.99 ± 0.49	2.89 ± 0.12	3.00 ± 0.75	2.85 ± 0.68	2.62 ± 0.68
	Fold change (Wy/CTL)		1.78		1.59		1.52		1.79		1.78		1.10		0.92		1.06		1.04		0.92	
	AF25#7	*Mus musculus* major urinary protein 2, MUP II (891 bp)	7.25 ± 1.94	6.50 ± 0.64	10.21 ± 1.52	5.82 ± 0.30	8.86 ± 0.85	2.94 ± 0.23	6.32 ± 0.98	0.70 ± 0.72	4.58 ± 0.12	0.06 ± 0.01	4.87 ± 1.43	5.02 ± 1.68	5.21 ± 0.15	5.30 ± 0.46	5.51 ± 1.12	5.51 ± 1.79	2.96 ± 0.11	3.39 ± 0.33	4.47 ± 0.37	5.04 ± 0.10
	Fold change (Wy/CTL)		0.90		0.57		0.33		0.11		0.01		1.03		1.02		1.00		1.15		1.13	

Values represent mean ± S.D. '+' represents the presence of a band (cDNA) on fluoroDD gel or Northern blot, while '-' represents the corresponding band (cDNA) was not observed on the fluoroDD gel or Northern blot. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. The signal intensity of a band on Northern blot was quantified by MultiAnalyst.

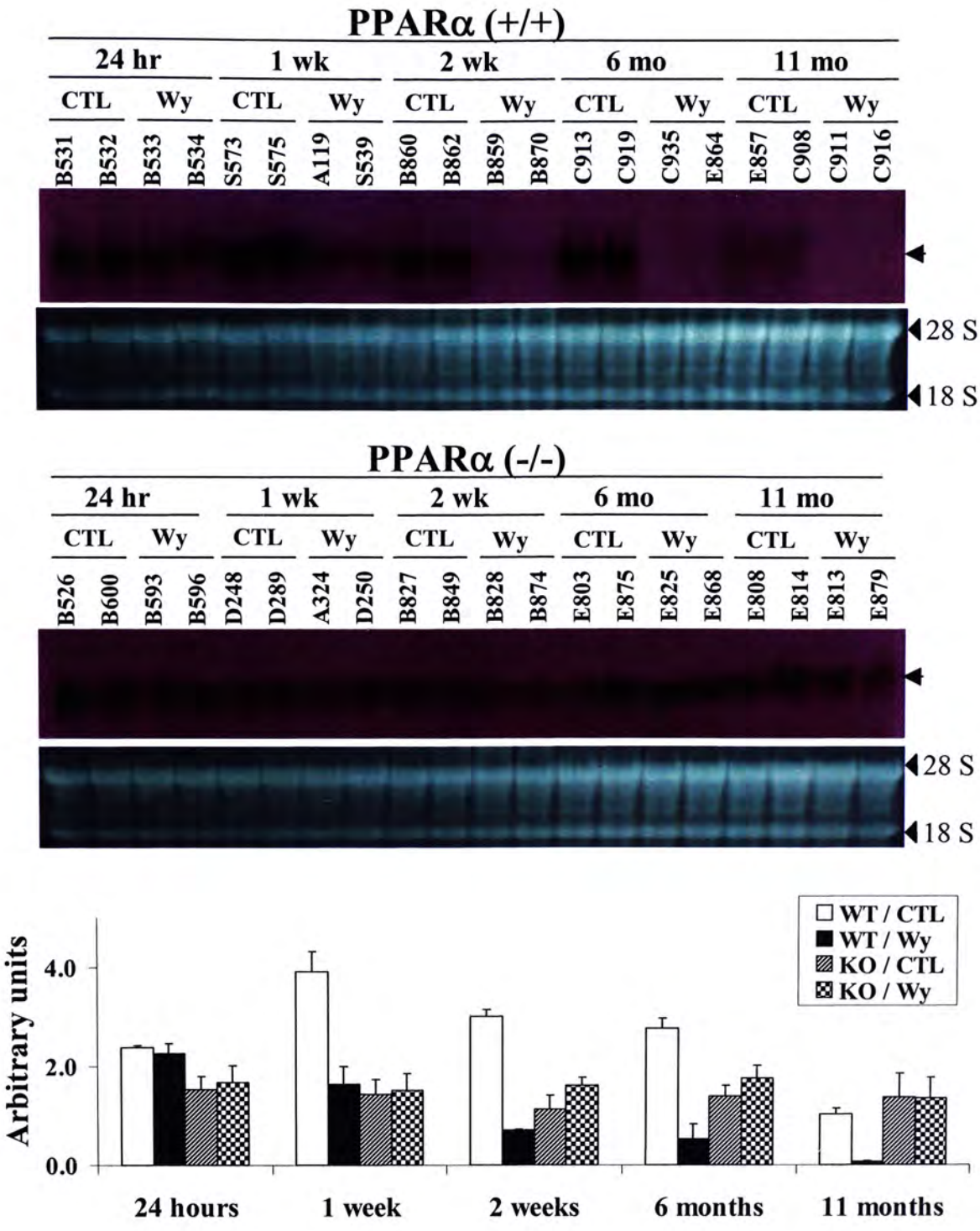

Figure 3.11.32. Temporal expression of AJ1#5 (carboxylesterase). Total liver RNA (30 μg) of two mice of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week, 2 weeks, 6 months and 11 months treatment were separated on 1% agarose-formaldehyde gels and transferred to nylon membranes. The membranes were then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents.

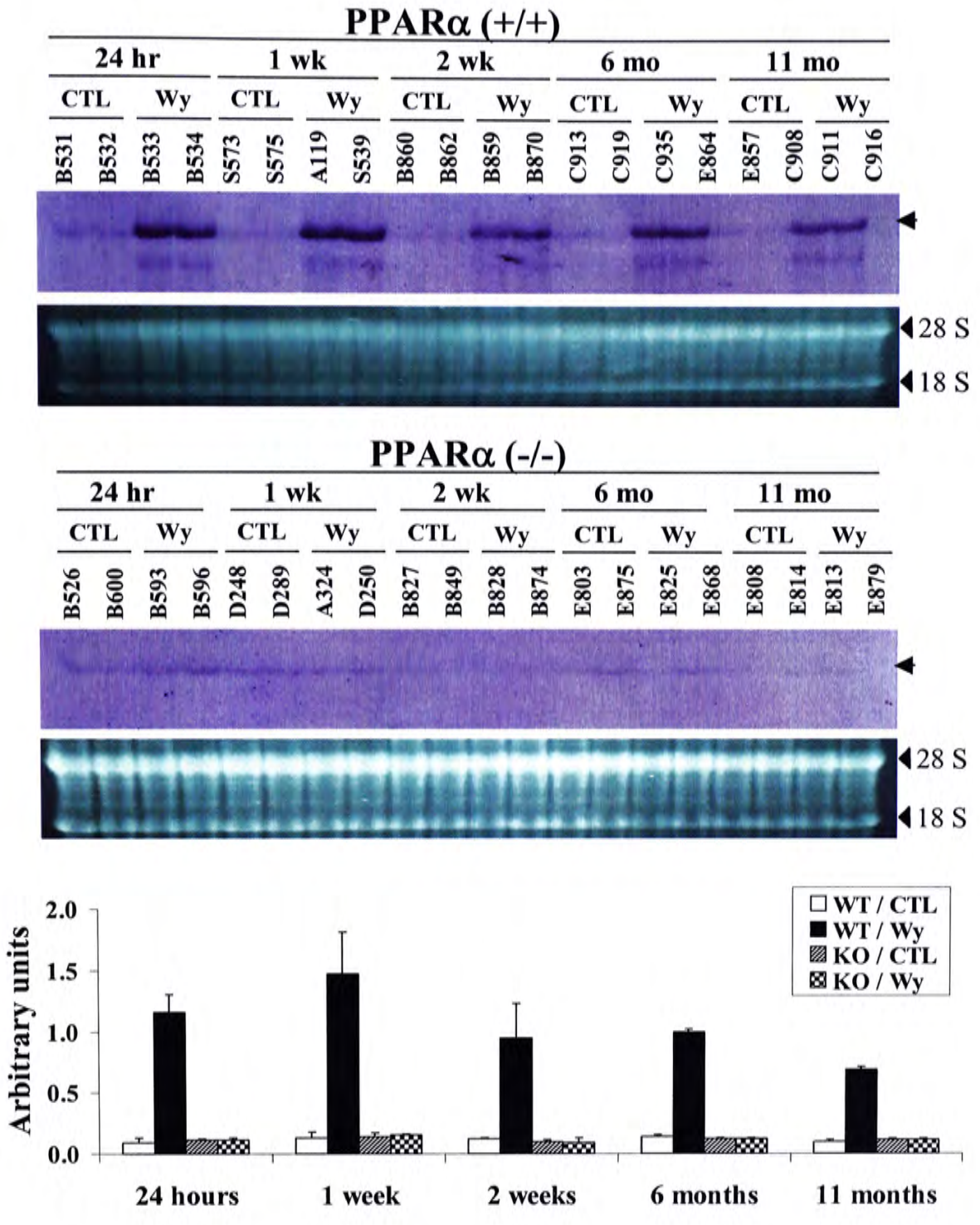

Figure 3.11.33. Temporal expression of AJ2#10 (peroxisomal acyl-CoA oxidase). Total liver RNA (30 μg) of two mice of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week, 2 weeks, 6 months and 11 months treatment were separated on 1% agarose-formaldehyde gels and transferred to nylon membranes. The membranes were then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents.

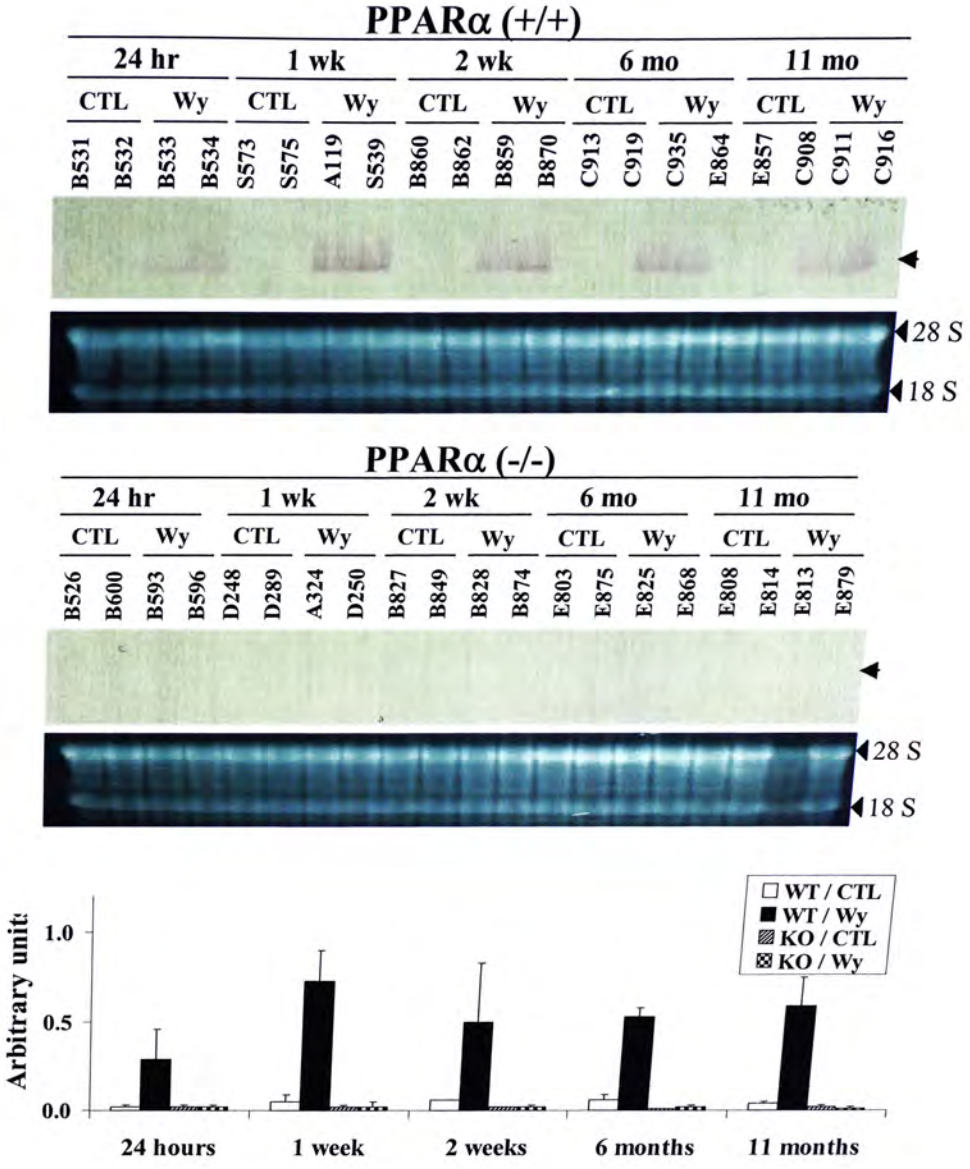

Figure 3.11.34. Temporal expression of AL3#3 (hydroxysteroid (17-beta) dehydrogenase 11). Total liver RNA (30 μg) of two mice of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week, 2 weeks, 6 months and 11 months treatment were separated on 1% agarose-formaldehyde gels and transferred to nylon membranes. The membranes were then hybridized with a DIG-labeled RNA probe and signals were detected by colorimetric method with NBT and BCIP reagents.

Table 3.11.10. Summary of Northern blot analysis on temporal expression patterns of cDNA fragments subcloned from gels AJ (AP6 and ARP14) and AL (AP7 and ARP15)

Gel	Subclone I.D.	Gene homology (Transcript size)	Northern blot densitometry reading (arbitrary units)																			
			PPARα (+/+)										PPARα (-/-)									
			24 hr		1 wk		2 wk		6 mo		11 mo		24 hr		1 wk		2 wk		6 mo		11 mo	
			CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy
AJ (AP6 & ARP14)	AJ1#5	Mouse carboxylesterase, Carboxylesterase (2006 bp)	2.39 ± 0.04	2.27 ± 0.19	3.92 ± 0.42	1.64 ± 0.37	3.02 ± 0.13	0.71 ± 0.01	2.77 ± 0.21	0.53 ± 0.31	1.03 ± 0.06	0.06 ± 0.02	1.52 ± 0.28	1.69 ± 0.34	1.43 ± 0.32	1.51 ± 0.35	1.13 ± 0.28	1.61 ± 0.17	1.39 ± 0.22	1.77 ± 0.25	1.37 ± 0.49	1.35 ± 0.43
	Fold change (Wy/CTL)		0.95		0.42		0.23		0.19		0.06		1.11		1.06		1.42		1.28		0.98	
	AJ2#10	*Mus musculus* peroxisomal acyl-coA oxidase, AOX (3778 bp)	0.09 ± 0.04	1.16 ± 0.14	0.13 ± 0.05	1.47 ± 0.34	0.12 ± 0.01	0.95 ± 0.28	0.14 ± 0.01	1.00 ± 0.02	0.10 ± 0.01	0.69 ± 0.02	0.11 ± 0.01	0.11 ± 0.02	0.14 ± 0.03	0.15 ± 0.01	0.10 ± 0.01	0.09 ± 0.04	0.12 ± 0.01	0.12 ±0.01	0.11 ± 0.01	0.11 ± 0.01
	Fold change (Wy/CTL)		12.89		11.31		7.92		7.14		6.90		1.00		1.07		0.90		1.00		1.00	
AL (AP7 & ARP15)	AL3#3	*Mus musculus* hydroxysteroid (17-beta) dehydrogenase 11, HSD17β11 (1713 bp)	0.02 ± 0.00	0.34 ± 0.24	0.05 ± 0.04	0.73 ± 0.17	0.06 ± 0.00	0.77 ± 0.23	0.06 ± 0.03	0.64 ± 0.11	0.04 ± 0.01	0.60 ± 0.12	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.03	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01
	Fold change (Wy/CTL)		17.00		14.6		12.83		10.67		15.00		1.00		1.00		1.00		1.00		1.00	

Values represent mean ± S.D. '+' represents the presence of a band (cDNA) on fluoroDD gel or Northern blot, while '-' represents the corresponding band (cDNA) was not observed on the fluoroDD gel or Northern blot. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. The signal intensity of a band on Northern blot was quantified by MultiAnalyst.

months. Maximum induction was found at 1 week 0.1% Wy-14,643 treatment. In PPARα (-/-) mice fed with the 0.1% Wy-14,643 diet, no induction was found after short-term and long-term exposure.

Fragment AO1#2 [mouse adipose differentiation related protein (ADRP) (Figure 3.11.35) (Table 3.11.11)] was up-regulated in 0.1% Wy-14,643 treated PPARα (+/+) mice. The up-regulation was started at 24 hours and consistently induced to 11 months. No induction was observed in the PPARα (-/-) mice fed with the 0.1% Wy-14,643 diet after short-term and long-term treatment.

Up-regulation of AO2#8 [mouse carnitine O-octanoyltransferase (Crot) (Figure 3.11.36) (Table 3.11.11)] was found in 0.1% Wy-14,643 treated PPARα (+/+) mice. The up-regulation of AO2#8 was started at 24 hours and continued to 11 months treatment. In PPARα (-/-) mice fed with the 0.1% Wy-14,643 diet, no induction was found after short-term and long-term exposure.

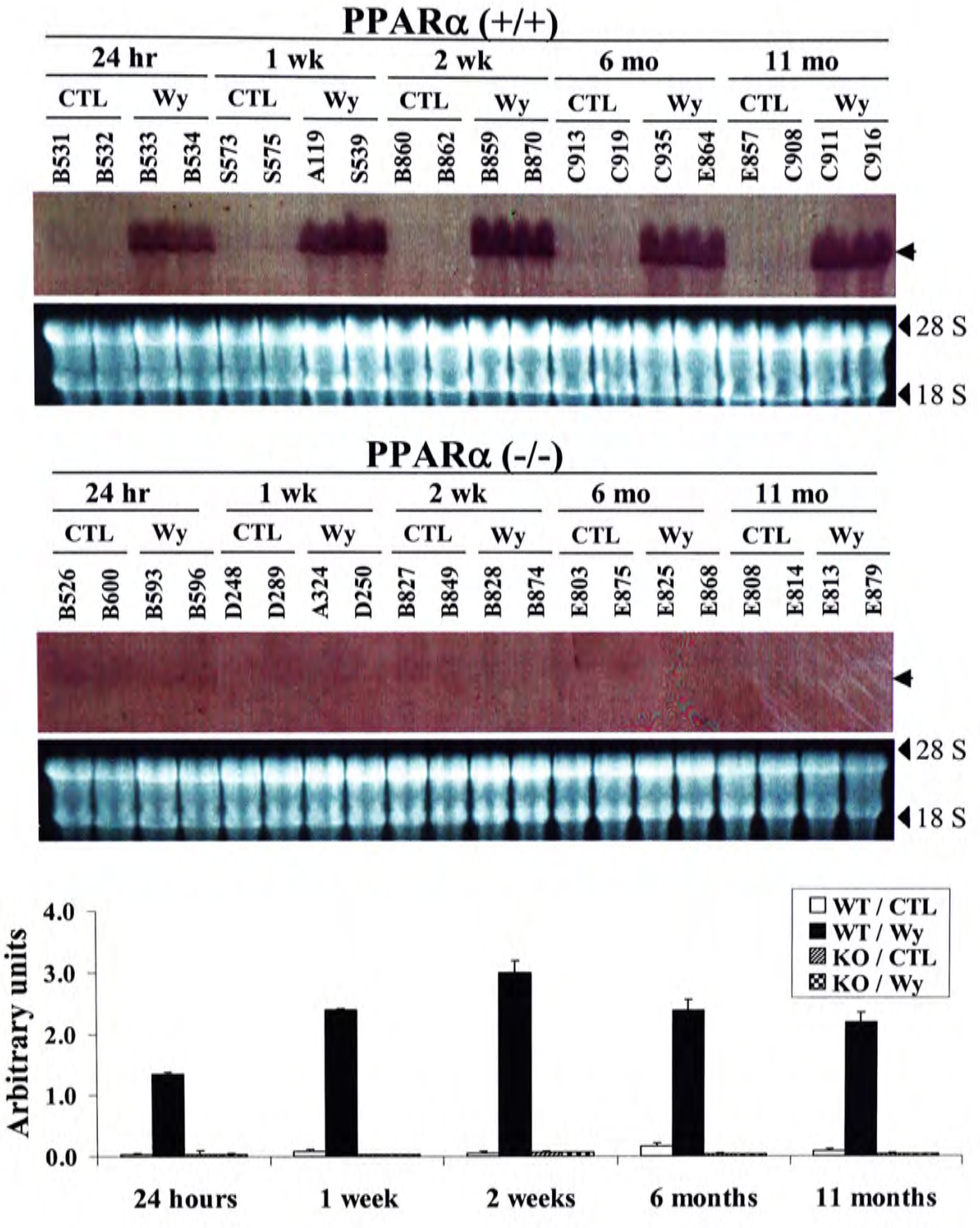

Figure 3.11.35. Temporal expression of AO1#2 (adipose differentiation related protein). Total liver RNA (30 μg) of two mice of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week, 2 weeks, 6 months and 11 months treatment were separated on 1% agarose-formaldehyde gels and transferred to nylon membranes. The membranes were then hybridized with a PCR DIG-labeled cDNA probe and signals were detected by colorimetric method with NBT and BCIP reagents.

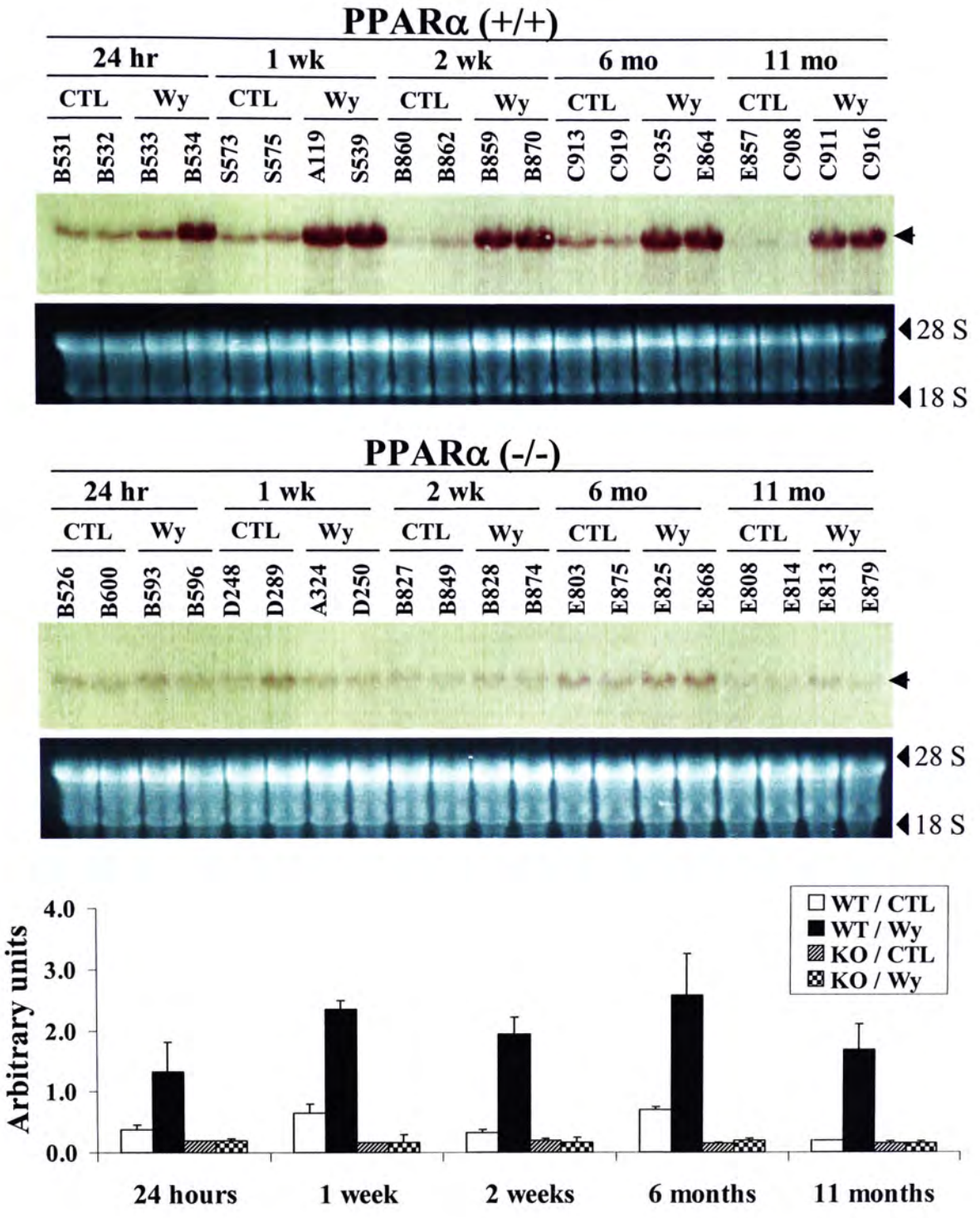

Figure 3.11.36. Temporal expression of AO2#8 (carnitine O-octanoyltransferase). Total liver RNA (30 μg) of two mice of PPARα (+/+) and PPARα (-/-) fed with a 0.0% control (CTL) or 0.1% (w/w) Wy-14,643 (Wy) diet for 24 hours, 1 week, 2 weeks, 6 months and 11 months treatment were separated on 1% agarose-formaldehyde gels and transferred to nylon membranes. The membranes were then hybridized with a PCR DIG-labeled cDNA probe and signals were detected by colorimetric method with NBT and BCIP reagents.

Table 3.11.11. Summary of Northern blot analysis on temporal expression patterns of cDNA fragments subcloned from gel AO (AP5 and ARP10)

Gel	Subclone I.D.	Gene homology (Transcript size)	Northern blot densitometry reading (arbitrary units)																			
			PPARα (+/+)										PPARα (-/-)									
			24 hr		1 wk		2 wk		6 mo		11 mo		24 hr		1 wk		2 wk		6 mo		11 mo	
			CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy	CTL	Wy
AO (AP5 & ARP10)	AO1#2	Mouse adipose differentiation related protein, ADRP (1680 bp)	0.03 ± 0.02	1.34 ±0.09	0.08 ± 0.03	2.40 ± 0.01	0.05 ± 0.03	2.99 ± 0.20	0.17 ± 0.04	2.38 ± 0.18	0.08 ± 0.04	2.19 ± 0.16	0.04 ± 0.05	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.06 ± 0.02	0.06 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
	Fold change (Wy/CTL)		44.67		30.00		59.80		14.00		27.38		1.00		1.00		1.00		1.00		1.00	
	AO2#8	*Mus musculus* carnitine O-octanoyl-transferase, Crot (2844 bp)	0.38 ± 0.08	1.32 ± 0.49	0.64 ± 0.15	2.35 ± 0.14	0.32 ± 0.06	1.95 ± 0.27	0.70 ± 0.04	2.57 ± 0.69	0.19 ± 0.01	1.69 ± 0.42	0.19 ± 0.01	0.20 ± 0.03	0.16 ± 0.01	0.16 ± 0.01	0.19 ± 0.04	0.16 ± 0.08	0.14 ± 0.02	0.19 ± 0.04	0.15 ± 0.03	0.15 ± 0.03
	Fold change (Wy/CTL)		3.47		3.67		6.09		3.67		8.89		1.05		1.00		0.84		1.36		1.00	

Values represent mean ± S.D. '+' represents the presence of a band (cDNA) on fluoroDD gel or Northern blot, while '-' represents the corresponding band (cDNA) was not observed on the fluoroDD gel or Northern blot. The relative intensity of the gene expression among the four treatment groups is indicated by the number of '+'. The signal intensity of a band on Northern blot was quantified by MultiAnalyst.

Chapter 4 Discussion

4.1 Body weight changes

There was significant decrease in body weights of the PPARα (+/+) mice after chronic exposure (6 and 11 months) of the Wy-14,643 diet. Decrease in body weights of rodents after Wy-14,643 treatment were reported in previous studies (Biegel *et al.*, 1992; Peters *et al.*, 1997; Biegel *et al.*, 2001). These decrease in body weights were due to the hypolipidemic effect of the Wy-14,643 compound but not due to starvation of mice. Enough food was provided and food consumption of different treatment groups was more or less the same for both short-term and long-term treatment. In this project, we mentioned that the expression of genes that involved in lipid metabolism was enhanced after Wy-14,643 treatment. The up-regulation of genes that involved in lipid metabolism lead to the decrease of the body weight. Besides, the serum triglyceride level was decreased due to the hypolipidemic effect of Wy-14,643 compound. The combined effect of enhancement of lipid metabolism and triglyceride catabolism result in the loss of body weight in PPARα (+/+) mice. The decrease in body weights were not observed in PPARα (-/-) mice that fed with Wy-14,643 as the hypolipidemic effect is mediated by PPARα (Peters *et al.*, 1997).

High mortality rate that found in the 0.1% Wy-14,643 treated PPARα (+/+) mice was mentioned in the previous study (Peters *et al.*, 1997; Biegel *et al.*, 2001) and might be due to the liver enlargement and probably liver cancer. Besides, no mice died in the other treatment groups during the 6 and 11 months treatment suggested that the high mortality rate was Wy-14,643-induced and mediated by PPARα.

4.2 Organ weight changes

Hepatomegaly was observed in PPARα (+/+) mice after short term (1 week) exposure with the 0.1% Wy-14,643 diet and the level of hepatomegaly was increased during the chronic administration of Wy-14,643. Liver enlargement of rodent induced after acute and chronic exposure of Wy-14,643 was reported in previous studies (Marsman *et al.*, 1988; Crunkhorn *et al.*, 2004). As hepatomegaly was not observed in PPARα (-/-) mice fed with the 0.1% Wy-14,643 diet for both short-term and long-term treatment, it indicated that the enlargement of liver was mediated by PPARα (Lee *et al.*, 1995; Peters *et al.*, 1997). Liver enlargement in Wy-14,643-treated PPARα (+/+) is due to the increase in cell replication and size of the hepatocytes (Carthew *et al.*, 1998; Corton *et al.*, 2000).

Relative white fat and brown fat weights were decreased in the 0.1% Wy-14,643 treated PPARα (+/+) mice started at 2 weeks treatment. However, these

were not observed in the PPARα (-/-) mice for short-term and long-term Wy-14,643 treatment. The decrease in the white fat and brown fat support the hypolipidemic effect of Wy-14,643 compound. Genes that involved in the lipid metabolism were up-regulated after Wy-14,643 treatment. With the enhancement of lipid catabolism, fat that stored in white fat and brown fat will be burnt that lead to the decrease in sizes of white fat and brown fat.

Relative spleen weight was also decreased after 11 months treatment in PPARα (+/+) mice fed with the 0.1% Wy-14,643 diet. Significant atrophy of spleen after treatment with potent peroxisome proliferators such as perfluorooctanoic acid (PFOA), di(2-ethylhexyl)phthalate (DEHP), Wy-14643 and nafenopin was reported in the previous study (Yang *et al.*, 2000). Decrease in the number of splenocytes (about 50%) was greater than the decrease in spleen weight after treatment with PFOA. This suggested that the loss of splenocytes rather than the organ matrix. The decrease in no. of splenocytes might be due to enhancement of apoptotsis and/or decrease in cell proliferation. The decrease in size of spleen might be also due to the decrease in size of the splenocytes.

The relative organ weights of brain, heart and lung were higher in 0.1% Wy-14,643 fed PPARα (+/+) mice compared to controls. The body weights were decrease with the hypolipidemic effect of Wy-14,643, however, the sizes and absolute

weights of brain, heart and lung were not change. Hence, the relative organ weights of brain, heart and lung were higher when compared with the controls (David *et al.*, 2000).

4.3 Serum cholesterol and triglyceride levels

The level of serum cholesterol is significantly increased at 11 months 0.1% Wy-14,643-treated PPARα (+/+). However, this result was not matched to previous reported that serum cholesterol level was lowered with the administration of Wy-14,643 (Issemann and Green, 1990). Increase in serum cholesterol level induced by perfluorooctanoic acid (PFOA) was mentioned in a previous study (Xie *et al.*, 2003). The serum cholesterol level was first decreased by PFOA but increase after seventh day of treatment. Xie *et al.* suggested that the increase in serum cholesterol level might be due to other changes caused by peroxisome proliferators.

Serum triglyceride level of PPARα (+/+) mice treated with 0.1% Wy-14,643 was decreased in all the different time points (24 hours, 1 week, 2 weeks, 6 months and 11 months). The decrease in serum triglyceride level is due to the hypolipidemic effect of Wy-14,643 (Issemann and Green, 1990). The hypolipidemic drugs enhance the triglyceride catabolism by suppressing the ApoCIII and induced the activities of lipoprotein lipase which lead to the induction of lipoprotein lysis (Staels *et al.*, 1997).

4.4 Liver histology

Abnormal hepatocytes arrangement and enlarged sinusoidal spaces were observed only in the 0.1% Wy-14,643-treated PPARα (+/+) mice and started at 1 week treatment. Trabecular cell clusters, hepatocytes without nucleus or dark nucleus and invasion of blood was found in 0.1% Wy-14,643-treated PPARα (+/+) mice after 11 months treatment and all of these are the characteristics of tumor cells. These features and severe liver damages were not observed in other treatment groups. Hepatocarcinoma caused by chronic exposure of Clofibrate and Wy-15,643 to mice were reported in previous studies (Reddy and Qureshi, 1979; Peters *et al.*, 1997).

Lipid accumulation was observed in both the control and treatment groups of PPARα (-/-) mice and Wy-14,643 treated PPARα (+/+) mice. The hepatic lipid accumulation in the PPARα (-/-) mice may be due to inhibition mitochondrial fatty acid import (Gervois *et al.*, 2000). The lipid accumulation of Wy-14,643 treated PPARα (+/+) mice at 2 weeks, 6 months and 11 months may be due to the enhanced catabolism of lipid that induced by Wy-14,643. In my study, several PPARα-dependent and Wy-14,643-responsive genes that involved in lipid metabolism were confirmed. The obvious and early inductions of these genes support the PP-induced catabolism of lipid. The accumulation of find lipid droplets in the

report (Lalwani *et al.*, 1981; Gorgas and Krisans, 1989).

4.5 Functions and roles of identified PPARα-dependent and Wy-14,643-responsive genes

Total 17 different genes (12 up-regulated and 5 down-regulated) were identified to be PPARα-dependent (Table 4.5.1). Most of the genes identified are related to lipid metabolism, whereas the others are involved in peroxisome biogenesis, plasma triglyceride metabolism, xenobiotics metabolism, adipocyte differentiation or sterol carrier protein, and some are cancer marker genes.

Mouse peroxisomal delta3, delta2-enoyl-Coenzyme A isomerase (Peci) is involved in the β-oxidation of unsaturated fatty acids. Delta3, delta2-enoyl-Coenzyme A is found in both peroxisome and mitochondria. The breakdown of unsaturated fatty acid requires the enzymes that catalyze the isomerzation and reduction of double bonds (Kunau *et al.*, 1995). The peroxisomal delta3, delta2-enoyl-Coenzyme A is involved in the 3-cis→ 2-trans and 3-trans→ 2-trans activity (Zhang *et al.*, 2002). In this study, it showed that maximum induction of Peci was observed at 24 hours treatment. Induction of Peci was started at 24 hours and continued to 11 months in PPARα (+/+) mice fed with the 0.1% Wy-14,643 diet. However, this time-dependent induction was not observed in the

Table 4.5.1. List of PPARα regulated genes in liver (with the genes identified in this project)

Function of gene	Genes	
Adipose differentiation	Adipose differentiation related protein	
Amino acid metabolism	Alanine aminotransferase	Cysteine sulfinic acid decarboxylase function
	Alanine glyoxylate transaminase	Cytosolic aspartate aminotransferase
	Arginase	Glutaminase
	Arginino-succinate lyase	Hydroxypyruvate/ gloxylate reductase
	Arginino-succinate synthase	Mitochondrial aspartate aminotransferase
	Carbamoyl-phosphate synthase-I	Ornithine transcarbamoylase
Acute phase response	Alpha-1 acid glycoprotein/serine protease inhibitor	Fibrinogen
Bile acid metabolism	Cytochrome P450 7A1	
Bile salt transport	Na+ taurocholate cotransporting polypeptide	
Biotransformation	Aldehyde dehydrogenase type 3	Glutathione-S-transferase alpha
	Carboxyesterase	Cytochrome P450 3A11
	Cytochrome P450 2A5	UDP-glycuronosyltransferase 1A9
	Cytochrome P450 2C29	UDP-glucuronosyltransferase 2b5
	Cytosolic epoxide hydrolase	
Cell cycle	c-myc	Cyclin D1
Cell cycle regulation	G0/G1 switch gene 2	
Cellular proliferation	glucose-regulated protein-94	
Cholesterol metabolism	Cytochrome P450 12A1	Liver X receptor α
	Cytochrome P450 27A1	
Cholesterol transport	ABCA-a (via LXRα)	ABCG-8 (via LXRα)
	ABCG-5 (via LXRα)	
Desaturation of fatty acid	Delta-6-Desaturase	Stearoyl-CoA desaturase
	Delta-5-desaturase	
Fatty acid activation	Long-chain acyl-CoA synthetase	
Fatty acid binding/transport	Cytosolic fatty acid binding protein	
Fatty acid transport	Fatty acid transport protein-1	Fatty acid translocase
Fatty acid β-oxidation	Acyl-CoA oxidase	Medium-chain acyl-CoA dehydrogenase
	ATP-binding cassette half-transporter type 2	Long-chain acyl-CoA dehydrogenase
	ATP-binding cassette half-transporter type 3	Short-chain-specific 3-ketoacyl-CoA thiolase
	Bifunctional enzyme	Short-chain acyl-CoA
	Carnitine palmitoyl-transferase I	Sterol carrier protein-X
	Carnitine palmitoyl-transferase II	Thiolase B
	Carnitine O-octanoyltransferase	Very long chain acyl-CoA dehydrogenase
	Dodecenoyl-CoA delta-isomerase	Very-long-chain acyl-CoA synthetase
	delta3, delta2-enoyl-Coenzyme A isomerase	
Fatty acid ω-hydroxylation	Cytochrome P450 4A1	Cytochrome P450 4A10
	Cytochrome P450 4A3	Cytochrome P450 4A14
	Cytochrome P450 4A6-Z	
Fatty acid synthesis	Malic enzyme	

Table 4.5.1. List of PPARα regulated genes in liver (with the genes identified in this project) (con't)

Function of gene	**Genes**	
Fatty acyl-CoA ester transport	Acyl-CoA binding protein	
Gluconeogenesis	Lactate dehydrogenase A4	
Glucose oxidation	Pyruvate dehydrogenase kinase isoform 4	
Heme biosynthesis	Coproporphyrinogen oxidase	
Hepatic sterol	Farnesoid X receptor/Bile acid receptor	
Hepatobiluary lipid transport	Multidrug resistance 2	
Hydrolysis of Acyl-CoA	Cytosolic acyl-CoA thioesterase-I	Peroxisomal acyl-CoA thioesterase 1A
	Cytosolic acyl-CoA thioesterase-II	Peroxisomal acyl-CoA thioesterase 1B
	Mitochondrial acyl-CoA thioesterase-I	Peroxisomal acyl-CoA thioesterase 2
Iron-binding protein	Lactoferrin	
Inflammation/ apoptosis	IκBα	
Iron transport	Transferrin	
Ketogenesis	Mitochondrial HMG-CoA synthase	Mitochondrial HMG CoA synthase
Oxygen-free radical metabolism	Superoxide dimutase	
Peroxisome biogenesis	Peroxisome biogenesis factor 16	
Plasma HDL metabolism	Apolipoprotein-AI	Apolipoprotein-AV
	Apolipoprotein-A I (via rev-erbα)	Apolipoprotein-CIII
	Apolipoprotein-AII	Phospholipid transfer protein
	Apolipoprotein-A IV	Scavenger receptor B-type I
Pheromone carrier	Alpha-2 urinary globulin/MUP-I	
Repressor of gene transcription	Rev-erbα	
ROS production	Uncoupling protein-2	
Triglyceride clearance	Lipoprotein lipase	
Steroid metabolism	11-beta-Hydroxysteroid dehydrogenase I	17-beta-hydroxysteroid dehydrogenase XI
	17-beta-hydroxysteroid dehydrogenase IV	
Steroid hydroxylase	Cytochrome P450 2C11	Cytochrome P450 2C12
Vascular inflammation	Interleukin-6	Interleukin-6 receptor
	Interleukin-1 recpetor-type 1	

Genes that highlighted were confirmed in this study.

controls. This suggested that the induction of Peci was mediated by PPARα and Wy-14,643-responsive. Up-regulation of Peci was reported in 2 weeks Wy-14,643 treatment (Cherkaoui-Malki *et al.*, 2001). Up-regulation of the genes involved in peroxisomal β-oxdiation was the effect of peroxisome proliferators. The induction of Peci increase peroxisomal β-oxidation which will increase the production of H_2O_2. Imbalance between production and catabolism of H_2O_2 will lead to oxidative stress, which is one of the proposed mechanisms of PP-induced hepatocarcinogenesis.

Mouse apolipoprotein A-V (ApoA5) is suggested to be involved in regulations of plasma triglyceride levels. Human ApoA5 is induced with the administration of Wy-14,643 and this induction is associated with suppression of plasma triglyceride (Vu-Dac *et al.*, 2003). However, mouse ApoA5 that identified in this project is down-regulated with the chronic exposure of Wy-14,643. This species difference in gene regulation between human and rodents in response to peroxisome proliferators treatment is also observed for ApoA1 and ApoA2 (Desvergne and Wahli, 1999). ApoA1 and ApoA2, which are major HDL apolipoproteins and PPARα mediated, are down-regulated in rodents but up-regulated in humans. The reason for difference in species gene regulation of ApoA1, ApoA2 and ApoA5 is still not known. PPRE is identified in the human ApoA5 genes and human ApoA5 is believed as a positive and a direct target gene of PPARα activators (Desvergne and Wahli, 1999; Vu-Dac *et al.*,

2003). Identification of PPRE in ApoA5 will help to explain its role in plasma triglyceride regulation in rodent and also the difference in species gene regulation between human and rodent.

Mouse medium-chain acyl-CoA dehydrogenase (MCAD; C_4-C_{12}) catalyses the formation of 2-enoyl-CoA from the fatty acyl-CoA (Eaton *et al.*, 1996). PPARα regulates the mitochondrial β-oxidation by modulating the expression of medium-chain acyl-CoA dehydrogenase (MCAD) gene (Gulick *et al.*, 1994). The substrates for MCAD included medium-chain length acyl-CoA thioesters derived from medium-chain fatty acids that enter mitochondria by diffusion, products of mitochondrial β-oxidation of saturated and unsaturated long-chain fatty acids and products of peroxisomal β-oxidation of long-chain and very long-chain fatty acids. The mRNA expression of MCAD is predominantly in the tissue that use fatty acids as energy substrates, such as liver and heart (Kelly *et al.*, 1989). Induction of MCAD was observed started at 24 hours and persisted to 11 months in Wy-14,643-treated PPARα (+/+) mice. The induction of MCAD was PPARα-mediated as such induction was not observed in Wy-14,643-treated PPARα (-/-) mice. The induction of MCAD leads to the increase in mitochondrial β-oxidation.

Mouse Cyp4a10 and Cyp4a14 belong to the Cyp4a family (Cyp4a). Both Cyp4a10 and Cyp4a14 were up-regulated in PPARα (+/+) mice after 24 hours 0.1%

Wy-14,643 treatment and the inductions were persisted to 11 months. Such inductions were not found in PPARα (-/-) mice suggested that the mRNA expressions of Cyp4a10 and Cyp4a14 were PPARα-dependent (Lee *et al.*, 1995). The cytochrome monoxygenase system plays a central role in the oxidation of a wide variety of endogenase as well as exogenous compounds. The Cyp4a enzymes participate in this system as a distinct group of the cytochrome P450 superfamily. They catalyze the ω-hydroxylation of fatty acids and eicosanoids and are induced by fibrates and other peroxisome proliferators in liver and kidney. The first step of ω-hydroxylation generates ω-hydroxy fatty acid which is then dehydrogenated to a dicarboxylic acid in the cytosol (Johnson *et al.*, 1996; Reddy and Hashimoto, 2001). The dicarboxylic acid is then converted to its CoA derivative which can further metabolized via peroxisomal β-oxidation. The increase in substrates for peroxisomal β-oxidation will lead to oxidative stress. ω-hydroxylation of fatty acids can also generate superoxide and/or H_2O_2 as the rate of NADPH consumption increases. PPARα can provide a negative feedback on the intracellular levels of an endogenous ligand and PPARα may have an important role in the detoxification of some xenobiotics (Desvergne and Wahli, 1999).

Mouse peroxisomal bifunctional enzyme (PBFE) is also named enoyl-CoA hydratase/$_L$-3-hydroxyacyl-CoA dehydrogenase. It is induced by Wy-14,643 in

PPARα (+/+) mice. Consistent induction of PBFE was observed at 24 hours and until 11 months treatment. No induction of PBFE in PPARα (-/-) mice for both short-term and long-term Wy-14,643 treatment (Lee *et al.*, 1995). PBFE is involved in the $_{L}$-hydroxy-specific peroxisome proliferator-inducible β-oxidation system while the $_{D}$-PBE is involved in the $_{D}$-hydroxy-specific β-oxidation system (Qi *et al.*, 1999). PBFE acts on long- and very long-straight chain acyl-CoAs, long-chain dicarboxylyl-CoAs and the CoA esters of prostaglandins whereas the non-inducible $_{D}$-3-hydroxy-specific peroxisomal β-oxidation system is for branch chain acyl-CoA (Figure 4.5.1.1). The induction of PBFE expression and functional PPRE in PBFE are reported in previous studies (Zhang *et al.*, 1992; Bardot *et al.*, 1993).

Mouse very-long-chain acyl-CoA synthetase (VLCAS) is involved very long-chain fatty acid metabolism. Induction of VLCAS was observed in PPARα (+/+) mice fed with 0.1% Wy-14,643 and started at 24 hours. The induction was persisted to 11 months treatment and maximum induction was observed at 1 week treatment. VLCAS was up-regulated by Wy-14,643 and PPARα-dependent (Fischer *et al.*, 2003). Very long-chain fatty acid (VLCFA; ≥C22:0) can be β-oxidized predominantly in peroxisome. VLCFA is first activated to VLCFA-CoA in the β-oxidation. This reaction is catalyzed by very long-chain acyl-CoA synthetase (VLCAS) which is found in peroxisomes and microsomes but not in mitochondria

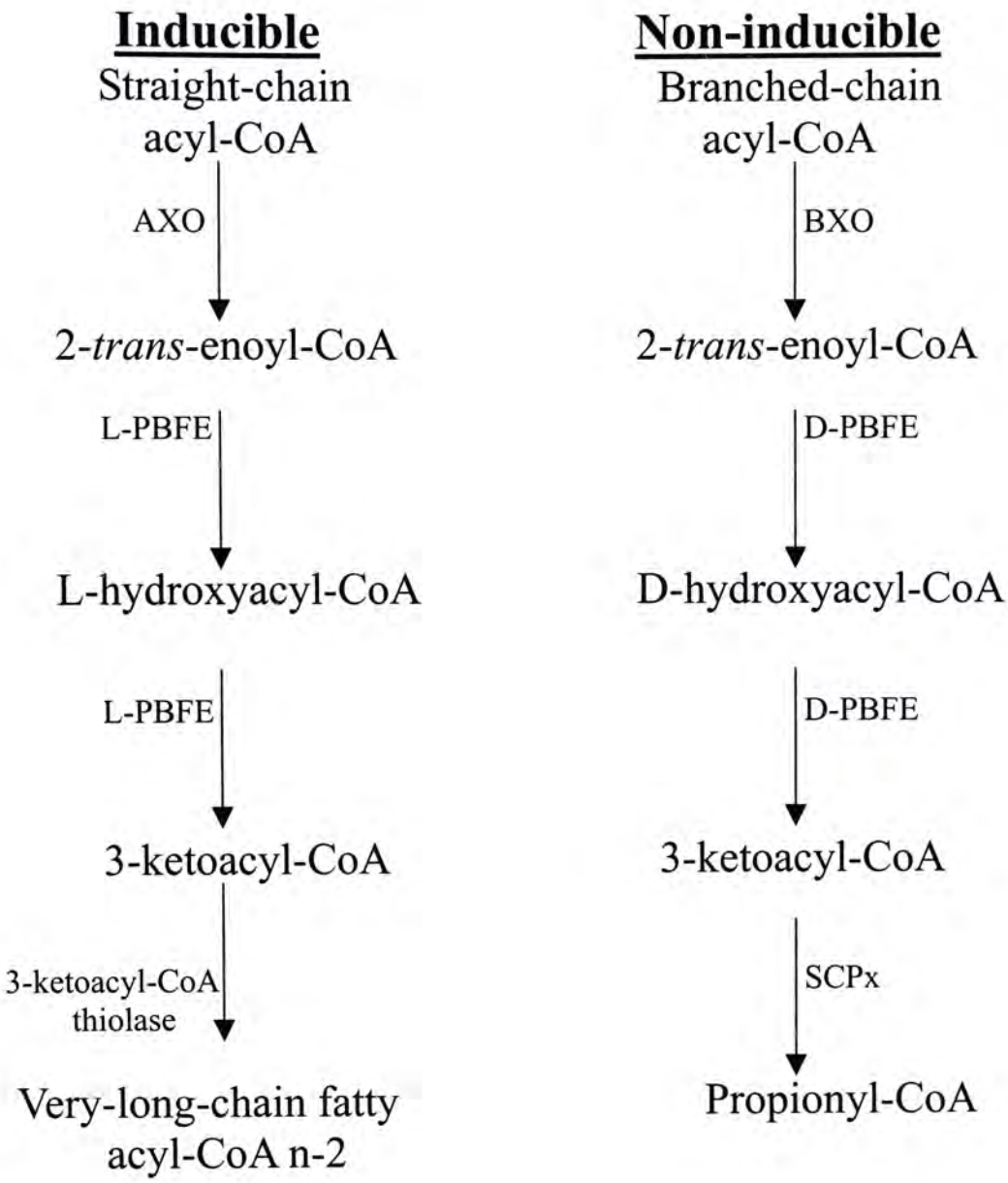

Figure 4.5.1.1. Peroxisome β-oxidation systems, inducible and non-inducible.

(Kalish *et al.*, 1995). However, rat liver mitochondria can β-oxidize hexacosanoyl CoA but not hexacosanoic acid (C26:0), whereas peroxisome can β-oxidize both substrates. It suggests that mitochondria can also β-oxidize VLFAs, provided that it is first converted to their CoAs by VLCAS (Singh *et al.*, 1987).

Mouse peroxisomal acyl-CoA oxidase (AOX) is the rate-limiting step of the pathway that show 10- to 30- fold induction with the administration of the peroxisome proliferators. The induction of peroxisomal fatty acid oxidation is important in protecting the liver against FFA toxicity as steatohepatitis is found in acyl-CoA oxidase knock-out mice (Everett *et al.*, 2000). Early and obvious up-regulation of AOX was found in PPARα (+/+) mice fed with the 0.1% Wy-14,643 diet. Maximum induction was observed at 1 week and continued to 11 months treatment. Induction of AOX causes the increase production of H_2O_2 and oxidative stress when there is no sufficient catalase, which is involved in the detoxification of H_2O_2 (Reddy and Rao, 1989).

Up-regulation of mouse carnitine O-octanoyltransferase (Crot) was found in PPARα (+/+) mice for both short-term and long-term treatment with 0.1% Wy-14,643. Crot is one of the carnitine acyltransferases in rat liver (CAT, carnitine acetyltransferae; Crot, carnitine O-octanoyltransferase; CPT, carnitine palmitoyltransferase), which is markedly increased by administration of peroxisome proliferators such as DEHP

(Ozasa *et al.*, 1983). Crot is suggested to be a peroxisomal enzyme whereas CAT and CPT are mitochondrial enzymes. Recent studies suggested that Crot plays a role in the transport of peroxisomal β-oxidation products to mitochondria. CAT, Crot and CPT showed different substrate specificities as CAT reacts with short-chain acyl derivatives, and CPT reacts with medium-chain and long-chain acyl derivatives. Crot is believed to be most active with hexanoyl derivatives although it also catalyze the reaction with medium-chain length substrates and to be most active toward octanoyl derivatives (Miyazama *et al.*, 1983). Crot can also reactive with the substrates with C_2-C_{20}. Crot is suggested to play a role in pumping out the peroxisomal β-oxidation products (short- and medium-chain acyl-CoA's) to the cytosolic compartment by their conversion to carnitine derivatives.

Mouse cysteine sulfinic acid decarboxylase (CSAD) is suggested to be markers genes of heptocarcinogensis. CSAD synthesizes a precursor of taurine, which becomes a major component of bile, and a neurotransmitter in brain. CSAD is suggested to be important in both maintenance and growth of hepatocytes as it is found in normal liver and its expression was induced during liver regeneration (Kishimoto *et al.*, 1996). CSAD expression is induced during hepatocarcinogenesis in the precancerous liver and maintained its high expression afterward. However, the relationship between over-expression of CSAD and hepatocarcinogenesis is still not

known. Over-expression of CSAD may cause hepatocarcinogenesis as the action of CSAD may result in some actual carcinogenic substrates *in vivo* in addition to taurine. In contrast, the over-expression of CSAD may be due to hepatocarcinogenesis. Intrinsic oncogene may transcriptionally activated CSAD. Over-expression of CSAD may be also due to the increase in demand for the removal of carcinogenic substances from the liver that require more bile (Kishimoto *et al.*, 1996). In this study, CSAD was weakly induced in PPARα (+/+) fed with the 0.1% Wy-14,643 diet after 24 hours and persisted to 11 months treatment. PPARα (-/-) mice treated with the 0.1% Wy-14,643 diet did not show any induction when compared the controls. This suggested the regulation CSAD was mediated by PPARα. The over-expression of CSAD may be due to the demand for the removal of Wy-14,643 compound.

Mouse major urinary protein II (MUP II) that also called α2- urinary globulin (α2U) in rat is induced by the male-specific pulsatile release of growth hormone but repressed by inflammation. Obvious down-regulation of MUP II was observed started at 1 week Wy-14,643 treatment in PPARα (+/+). The down-regulation of MUP with chronic exposure to Wy-14,643 has been mentioned and MUP might serve as an early tumor marker during hepatocarcinogenesis of mouse (Anderson *et al.*, 1999).

Mouse UDP-glucuronosyltransferase 2 family member 5 (UGT2b5) belongs to

the superfamily UDP-glucuronosyltransferases (UGTs). Down-regulation of UGT2b5 was observed in PPARα (+/+) mice fed with the 0.1% Wy-14,643 diet after 2 weeks treatment and this down-regulation was not observed in the controls. UGTs are located in the endoplasmic reticulum and perform a key process in endobiotic and xenbiotic metabolism. UGTs enhance the eliminations of their substrates via bile, faeces and urine through catalyse the conversion of lipophilic molecules into more polar, and thus hydrophilic, glucuronides. UGTs substrates include phenolics, steroid hormones, carcinogens such as benzo(α)pyrene metabolites and drugs. Thus, UGTs is important in the detoxification of endogenous, dietary and clinically administered agents as well as environmentally present chemical, which have been reported to be carcinogenic (Strassburg *et al.*, 1999). Down-regulation of UGT in pre-malignant adenomatous hyperomatous hyperplasia of the liver and malignant hepatocellular carcinoma is reported. It is suggested that UGT may be involved in genoprotection, however, further studies should be carried out before used it as an early marker in cancer development (Tukey and Strassburg, 2000).

Peroxisome biogenesis factor 16 (Pex16) is involved in the membrane-assembly step of peroxisome biogenesis (Honsho *et al.*, 2002). Pex16 was up-regulated at 24 hours Wy-14,643 treatment in PPARα (+/+) mice. The up-regulation was persisted to 11 months treatment and was not found in the PPARα (-/-) mice fed with the 0.1%

Wy-14,643 diet after short-term or long-term treatment. The up-regulation of Pex16 may play a role in peroxisome proliferation. Pex16 is required for the import of certain matrix proteins. Over-expression of Pex 16 caused accumulation of enlarged peroxisomes (Subramani, 1998). Peroxisome proliferation, which means increase in both numbers and size of peroxisomes, is observed with the administration of peroxisome proliferators after short-term treatment, so the induction of Pex16 is responsible for the PP-induced peroxisome proliferation.

Mouse serine protease inhibitor, clade A, member 1a (SPI) is also named α−1 antitrypsin (ATT). SPI is an acute phase protein which is synthesized primarily in the liver. SPI is an important factor in controlling tissue damage by protease in inflammatory diseases for example atherosclerosis. The maintenance of balance between protease and antiprotease is important in preventing tissue damage induced by proteinases in the microenvironment of injury and inflammation where inflammatory cells tend to accumulate (Dichtl *et al.*, 2000). Down-regulation of SPI was found in PPARα (+/+) mice fed with the 0.1% Wy-14,643 diet after 1 week treatment. The down-regulation was not found in PPARα (-/-) mice fed with 0.1% Wy-14,643 suggested the regulation of SPI was mediated by PPARα. Down-regulation of SPI after chronic (13 weeks) exposure with Wy-14,643 is reported in previous study. The suppression of the gene may be due to the anti-inflammatory

effect in mice that elicit by Wy-14,643 which possibly through increased leukotrine B_4 catabolism or inhibition of IL-1-induced production of IL-6, prostaglandin and cycloocygenase-2 (Anderson *et al.*, 1999)

Mouse hydroxysteroid (17-beta) dehydrogenase 11 (Hsd17β11) is a member of the 17β-hydroxysteroid dehydrogenase. The function of human Hsd17b11 is involved in estrogen and androgen inactivation (Mindnich *et al.*, 2004). The substrates for Hsd17β including glucocorticoids, sex steroids, bile acids, fatty acids and branched chain amino acids (Shafqat *et al.*, 2003; Mindnich *et al.*, 2004). The Hsd17β11 may be important for the metabolization of toxic compounds (Chai *et al.*, 2003; Motojima, 2004). Up-regulation of Hsd17β11 was started at 1 week treatment and only found in PPARα (+/+) mice fed with the 0.1% Wy-14,643 diet. Another member of the 17β-hydroxysteroid dehydrogenase, Hsd17β4 that has the same functions as Hsd17b11 in human and rodents is induced by peroxiome proliferators (Corton *et al.*, 1996).

Mouse carboxyesterase plays a role in the metabolism of xenobiotics and endogenous lipids. Carboxyesterases are distributed in different tissues and present in several subcellular organelles, whereas the highest abundant of carboxyesterase is observed in liver microsomes, where they are loosely bound to the lumenal surface of the endoplasmic reticulum. They hydrolyze lipid substrates and thus may have

different lipid-metabolizing functions. Carboxyesterase was down-regulated after 1 week 0.1% Wy-14,643 treatment in PPARα (+/+) mice. Carboxyesterase is down-regulated with chronic exposure of Wy-14,643 and this suppression is observed between 1 and 5 weeks of exposure. The delayed response of carboxyesterase to Wy-14,643 treatment suggested that carboxyesterase regulation is not a direct effect of PP-regulated activity of PPARα. The down-regulation of carboxyesterase may be the secondary event after long-term exposure to high doses of peroxisome proliferators (Poole *et al.*, 2001).

Mouse adipose differentiation related protein (ADRP) was up-regulated by 0.1% Wy-14,643 through PPARα. The up-regulation of ADRP was observed started at 24 hours and maximum up-regulation was found at 2 weeks Wy-14,643 treatment. Expression of ADRP is induced by PPARα ligands (Liu *et al.*, 2003). ADRP is a lipid droplets-associated protein that stimulates lipid accumulation and lipid droplets formation in murine fibroblasts and is expressed early during adipose differentiation (Imamura *et al.*, 2002). ADRP expression is induced by long-chain fatty acids but such induction is stimulated by short-chain fatty acids. ADRP, which bound to the surface of lipid droplets, may serve as concentrate unesterified fatty acids. They are then converted to fatty acyl-CoA thioesters for subsequent cellular utilization (Serrero *et al.*, 2000).

However, not all PPARα-dependent and Wy-14,643 responsive fragments could be confirmed by Northern blot analysis. No differential display patterns (e.g. mitochondria) and detectable signals (e.g. EST) were observed. The up-regulation of the genes might be not so obvious that could be observed and confirmed by Northern blot analysis.

4.6 Mechanism of PP-induced hepatocarcinogenesis

Oxidative stress, inhibition of apoptosis and increase cellular proliferation are the mechanism suggested so far for the PP-induced hepatocarcinogenesis. However, the mechanism(s) of PP-induced hepatocarcinogenesis is/are still not confirmed. Identification of PPARα-dependent and Wy-14,643-responsive genes can help us to explain the mechanism of PP-induced hepatocarcinogenesis. The genes that confirmed in this project are mainly involved in the fatty acid β-oxidation which is suggested to increase the oxidative stress (Figure 4.6.1). Increase in oxidative stress will lead to DNA damage that is one the proposed PP-induced hepatocarcinogenesis.

The PPARα dependent and Wy-14,643 responsive genes that confirmed in this project and related to oxidative stress was summarized in Figure 4.6.1. Peroxisomal delta3, delta2-enoyl-Coenzyme A isomerase, peroxisomal bifunctional enzyme, very-long-chain acyl-CoA synthetase and peroxisomal acyl CoA oxidase are the genes

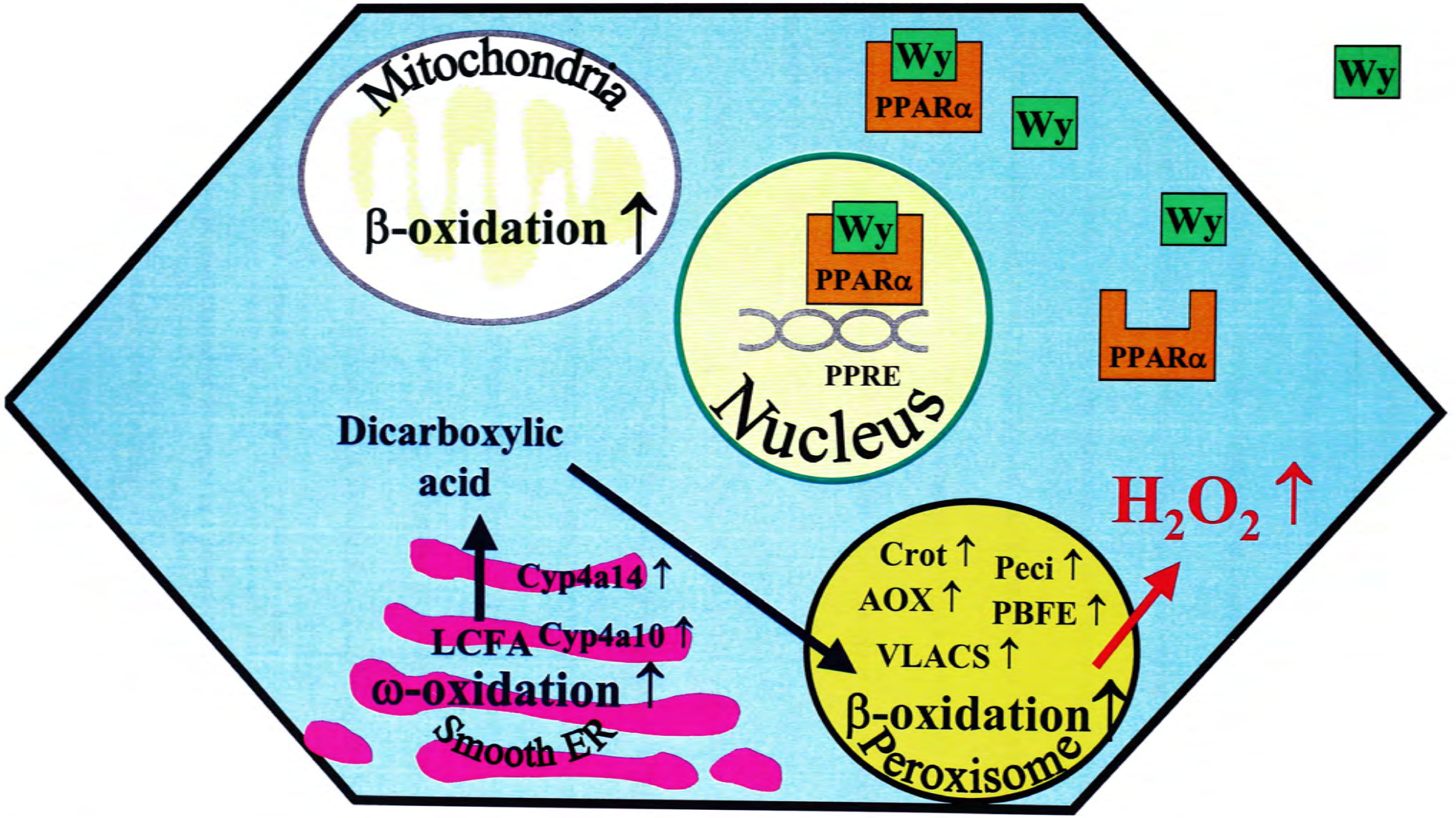

Figure 4.6.1. Summary of PPARα dependent and Wy-14,643 responsive genes caused oxidation stress.

that involved in peroxisomal β-oxidation. The over-expression of these genes especially acyl CoA oxidase leads to the induction of peroxisomal β-oxidation that in turns, increase the production of H_2O_2. However, as catalase that act on the H_2O_2 is only induced to 2- to 3-fold (Nemali *et al.*, 1989), excess H_2O_2 cannot be removed and cause oxidative stress. Besides, the increase in ω-hydroxylation increases the generation dicarboxylaic acids. Dicarboxylaic acids are one of the substrates for peroxisomal β-oxidation. The H_2O_2 generates by ω-hydroxylation and in turns of increasing substrates for peroxisomal β-oxidation also contribute to the increase in oxidative stress.

The alterations of gene expressions of all of the genes were observed after short-term Wy-14,643 treatment. Up-regulation of mouse peroxisomal delta 3, delta 2-enoyl-Coenzyme A isomerase, mouse acetyl-Coenzyme A dehydrogenase, medium chain, peroxisome biogenesis factor 16, mouse Cyp4a14, mouse peroxisomal bifunctional enzyme, mouse Cyp4a10, very-long-chain acyl-CoA synthetase, mouse peroxisomal acyl-CoA oxidase, mouse hydroxysteroid (17-beta) dehydrogenase 11, mouse adipose differentiation related protein and mouse carnitine O-octanoyltransferase were started at 24 hours and persisted to 11 months treatment while up-regulation of mouse cysteine sulfinic acid decarboxylase was started at 1 week and persisted to 11 months. Down-regulation of mouse serine protease

inhibitor 1-1, mouse major urinary protein II and mouse carboxyesterase was started at 1 week and continued to 11 months. Mouse UDP-glucuronosyltransferase 2 family member 5 was down-regulated at 2 weeks and persisted to 11 months. As the alteration of the genes observed after short-term treatment and persisted to cancer stage, these enable these genes to be cancer marker genes. Chemicals which induce these genes after short-term exposure to rodents may cause hepatocarcinogenesis after long-term exposure.

Charpter 5 Conclusion

Hepatomegaly, loss of body weight and hepatocarcinoma with trabecular patterns were observed in the 0.1% Wy-14,643 treated PPARα (+/+) mice after chronic exposure. Besides, relative organ weight of white fat, brown fat and spleen was lower in the 0.1% Wy-14,643 treated PPARα (+/+) mice compared with the controls.

Alterations of gene expression were also observed after 11 months treatment of Wy-14,643. Total 17 PPARα dependent and Wy-14,643 responsive genes were identified in this project. Peroxisomal delta3, delta2-enoyl-Coenzyme A isomerase, mouse cysteine sulfinic acid decarboxylase, mouse acetyl-Coenzyme A dehydrogenase, medium chain, peroxisome biogenesis factor 16, mouse Cyp4a14, mouse peroxisomal bifunctional enzyme, mouse Cyp4a10, very-long-chain acyl-CoA synthetase, mouse peroxisomal acyl-CoA oxidase, mouse hydroxysteroid (17-beta) dehydrogenase 11, mouse adipose differentiation related protein and mouse carnitine O-octanoyltransferase were confirmed to be up-regulated by 0.1% Wy-14,643 and PPARα dependent. Mouse apolipoprotein A-V, mouse UDP-glucuronosyltransferase 2 family member 5, mouse serine protease inhibitor 1-1, mouse major urinary protein II and mouse carboxyesterase were confirmed to be down-regulated by 0.1%

Wy-14,643 and PPARα dependent. Temporal expression of all the identified genes except apolipoprotein A-V were also performed.

Based on the genes identified, oxidative stress is suggested to be the mechanism of PP-induced hepatocarcinogenesis. However, whether the PP-induced hepatocarcinogenesis is induced by other mechanisms are not still clear. It is suggested that PP-induced hepatocarcinogenesis is due to the oxidative stress and also the effect of inhibition of apoptosis and increase in cell replication. Further studies are necessary to find out the relationship of different mechanisms and the chronic effect of peroxisome proliferators on humans.

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Appendix A. Tables of preparation of reaction mix

Table A1. Preparation of animal tail genotyping PCR reaction

Reagent	Stock conc.	Vol. per reaction (μl)	Final conc.
Nuclease-free water	-	19.125	-
10X PCR buffer	10X	2.5	1X
dNTP mix	10 mM	0.125	0.05 mM
Forward primer (F1)	10 μM	0.875	0.35 μM
Reverse primer (R3)	10 μM	0.875	0.35 μM
Taq polymerase	5 U/μl	0.5	0.1 U/μl
10-fold diluted DNA	-	1.0	-
Total	-	25	-

Table A2. Preparation of DNase I treatment

Reagent	Stock conc.	Vol. per reaction (μl)	Final conc.
$MgCl_2$	25 mM	40	10 mM
Tris-HCl (pH 7.5)	1 M	5	50 mM
RNase-free Dnase I	7.5 U/μl	4.2	0.3 U/μl
Total liver RNA	-	50.8	50 μg
Total	-	100	-

Table A3. Preparation of reverse transcription of non-fluoroDD and fluoroDD

Reagent	Stock conc.	Vol. per reaction (μl)	Final conc.
Nuclease-free water	-	7.8	-
Anchored primer (AP)	2 μM	2.0	0.2 μM
DTT	100 mM	2.0	10 mM
dNTP mix	250 μM	2.0	25 μM
Superscript™ II RT buffer	5X	4.0	1X
Superscript™ II RNase H⁻ reverse transcriptase	200 U/μl	0.2	2 U/μl
DNase I treated RNA	0.1 μg/μl	2.0	10 ng/μl
Total	--	20	-

Appendix A: Tables of preparation of reaction mix (Con't)

Table A4. Preparation of non-fluoroDD and fluoroDD RT-PCR

Reagent	Stock conc.	Vol. per reaction (μl)	Final conc.
Nuclease-free water	-	0.45	-
Anchored primer (AP)	5 μM	0.7	0.35 μM
Arbitrary primer (ARP)	2 μM	1.75	0.35 μM
dNTP mix	250 μM	2.0	50 μM
10X PCR buffer	10X	1.0	1X
Taq polymerase	5 U/μl	0.1	0.05 U/μl
RT mixture	-	4.0	-
Total	-	10	-

Table A5. Preparation of reamplification of differentially expressed cDNA fragments

Reagent	Stock conc.	Vol. per reaction (μl)	Final conc.
Nuclease-free water	-	8.2	-
10X PCR buffer	10X	2.0	1X
dNTP mix	250 μM	1.6	20 μM
M13 reverse primer	2 μM	2.0	0.2 μM
T7 promoter primer	2 μM	2.0	0.2 μM
Taq polymerase	5 U/μl	0.2	0.05 U/μl
Gel band elute	-	4.0	-
Total	-	20	-

Table A6. Preparation of PCR reaction for DNA sequencing

Reagent	Stock conc.	Vol. per reaction (μl)	Final conc.
10X Sequencing reaction buffer	10X	2	1X
dNTP mix	-	1	-
DdUTP Dye terminator	-	2	-
DdGTP Dye terminator	-	2	-
DdCTP Dye terminator	-	2	-
DdATP Dye terminator	-	2	-
M13 forward (-20) primer/ M13 reverse primer	1.6 μM	2	0.16 μM
Polymerase enzyme	-	1	-
DNA template	100 fmol	6	30 fmol
Total	-	20	-

Appendix A: Tables of preparation of reaction mix (Con't)

Table A7. Preparation of PCR reaction for RNA probe

Reagent	Stock conc.	Vol. per reaction (µl)	Final conc.
Nuclease-free water	-	5.4	-
10X PCR buffer	10X	2.0	1X
dNTP mix	250 µM	6.4	80 µM
Anchored primer (AP)	2 µM	2.0	0.2 µM
Arbitrary primer (ARP)	2 µM	2.0	0.2 µM
Taq polymerase	5 U/µl	0.2	0.05 U/µl
Plasmid DNA	1 ng/µl	2.0	2 ng/µl
Total	-	20	-

Table A8. Preparation of PCR reaction for cDNA probe

Reagent	Stock conc.	Vol. per reaction (µl)	Final conc.
Nuclease-free water	-	24.6	-
10X PCR buffer	10X	5.0	1X
PCR-DIG labeling mix	2 mM	5.0	200 µM
$MgCl_2$	25 mM	3.0	1.5 mM
Anchored primer (AP)	10 µM	5.0	1 µM
Arbitrary primer (ARP)	10 µM	5.0	1 µM
Taq polymerase	5 U/µl	0.4	0.04 U/µl
Plasmid DNA	20 ng/µl	2.0	0.8 ng/µl
Total	-	50	-

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 1.1: DNA sequence of cDNA subclone AA1#2 (AP1 & ARP2) using M13 forward (-20) primer

```
  1 GAATGTTACG ACTCACTATA GGGCGAATTG GGCCCTCTAG ATGCATGCTC GAGCGGCCGC
 61 CAGTGTGATG GATATCTGCA GAATTCGGCT TAGCGGATAA CAATTTCACA CAAGGAGCTA   ARP2*
121 GCATGGCGGC AGTGACCTGG AGTCGGGCTC GATGCTGGTG TCCGAGGTCT TCAGATTACA
181 AGTGGCTAAA CTGCACTTGG GCAGACCAAC AATGAGAGCC AGTCAGCAGG ACTTTGAAAA
241 TGCATTGAAC CAGGTGAAAC TCTTGAAGAA GGACCCGGGA AACGAAGTGA AGCTAAGACT
301 CTATGCACTG TACAAGCAGG CCACAGAAGG ACCCTGTAAT ATGCCTAAGC CTGGTATGCT
361 TGACTTTGTC AACAAAGCCA AGTGGGATGC ATGGAGTGCC CTGGGCAGCC TACCCAAGGA
421 AACTGCCAGG CAGAACTATG TGGATCTCGT ATCCAAAAAG AGTNCCTCAT CTGAAGCCNC
481 GAGCCAGGGA NAGCGTGGAG CTGATGAGAA AGCCGGGGAG TCCAAAGACA TNCTGNTAAC
541 TTCTGANGAT GGTATCACGA AGATCACGTT CANNCCGNCC NCCCAAAAGA NTGCCATATN
601 CTNNCAGATG TATCNNGATA TNATACTCGC CCTTAAAATG NCAGCNNNGA TACACTNTNA
661 TGCTGTTNNA CANGANCTNN GACACTACTG CNNNGGANGA CNGACTACNT NCTANTGCAG
721 G
```

B 1.2: Sequencing alignment of cDNA subclone **AA1#2** with **mouse peroxisomal delta 3, delta 2-enoyl-Coenzyme A isomerase (Peci)** by BLAST searching against the National Center for Biotechnology Information database

```
AA1#2      : 165 aggtcttcagattacaagtggctaaactgcacttgggcagaccaacaatgagagccagtc 224
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Peci: 296 aggtcttcagattacaagtggctaaactgcacttgggcagaccaacaatgagagccagtc 355

AA1#2      : 225 agcaggactttgaaaatgcattgaaccaggtgaaactcttgaagaaggacccgggaaacg 284
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Peci: 356 agcaggactttgaaaatgcattgaaccaggtgaaactcttgaagaaggacccgggaaacg 415

AA1#2      : 285 aagtgaagctaagactctatgcactgtacaagcaggccacagaaggaccctgtaatatgc 344
                 |||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Mouse Peci: 416 aagtgaagctaagactctatgcactgtacaagcaggccacagaaggaacctgtaatatgc 475

AA1#2      : 345 ctaagcctggtatgcttgactttgtcaacaaagccaagtgggatgcatggagtgccctgg 404
                 |||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||
Mouse Peci: 476 ctaagcctggtatgcttgactttgtcaacaaagccaagtgggatgcatggaatgccctgg 535

AA1#2      : 405 gcagcctacccaaggaaactgccaggcagaactatgtggatctcgtatccaaaaagagtn 464
                 |||||||||||||||||||||||||||||||||||||||||||||||||||||    ||||
Mouse Peci: 536 gcagcctacccaaggaaactgccaggcagaactatgtggatctcgtatccagtttgagtt 595

AA1#2      : 465 cctcatctgaagccncgagccaggganagcgtggagctgatgagaaagccggggagtcca 524
                 |||||||||||||| |||||||||| |||||||||||||||||||||||| ||||||||||
Mouse Peci: 596 cctcatctgaagccccgagccagggaaagcgtggagctgatgagaaagcccgggagtcca 655

AA1#2      : 525 aagacatnctgntaacttctgangatggtatcacgaagatcacgttcannccgnccnccc 584
                 ||||||| ||| |||||||||| |||||||||||||||||||||||||  | | || ||
Mouse Peci: 656 aagacatcctggtaacttctgaagatggtatcacgaagatcacgttcaatcggcccacca 715

AA1#2      : 585 aaaagantgccatatnctnncagatgtatcnngatatnatactcgcccttaaaa 638
                 |||||| |||||||| ||  |||||||||  |||||  |||||||| |||||||
Mouse Peci: 716 aaaagaatgccatatccttccagatgtatcgggatattatactcgcacttaaaa 769
```

B 1.3: Summary of sequence alignment of cDNA subclone **AA1#2** with **mouse Peci**

FluoroDD gel AA (AP1 + ARP2)						Subcloning		Sequencing		
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)	Subclone I.D.	Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AA1	-	+	-	-	1400	AA1#2	1400	M13 forward (-20)	*Mus musculus* peroxisomal delta 3, delta 2-enoyl-coenzyme A isomerase (Peci) (1581 bp)	0 447/474 (94%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 2.1: DNA sequence of cDNA subclone AA1#3 (AP1 & ARP2) using M13 forward (-20) primer

```
  1 TTACGACTCA CTATAGGGCG AATTGGGCCC TCTANNTGCA TGCTCGAGCG GCCGCCAGTG
 61 TGATGGATAT CTGCAGAATT CGGCTTAGCG GATAACAATT TCACACAAGG AGCTAGCATG   ARP2*
121 GCGGCAGTGA CCTGGAGTCG GGCTCGATGC TGGTGTCCGA GTGTCCTGCA GGTCTTCAGA
181 TTACAAGTGG CTAAACTGCA CTTGGGCAGA CCAACAATGA GAGCCAGTCA GCAGGACTTT
241 GAAAATGCAT TGAACCAGGT GAAACTCTTG AAGAAGGACC CGGGAAACGA ACGTGAAGCT
301 AAAGACTCTA TGCACTGTAC AAGCAGGCCA CAGNAAGGAC CCTGTAATAT GACACATNNA
361 NCNAAAAAAA AAACAAAANA ANNNNTACAC CNNAAGCCCA AGGTNGGGAT TGCATNGGGA
421 ATGCCCTGGG NAGCCTACCC AAAGGAAACT GCCAGGCAGA ACTNTGTGGA TCTCGTATCC
481 NGTTTGAGTT CCTCATCTGA AGCCCCGAGC CAGGGAAAGC GTGGAGCTGA TGAGAAAGCC
541 CNGGAGTCCN AAGACNTNCT GGTAACTTCT GAAGATGGTA TCCCGAAGAT CNNGTNCATT
601 CNGCCCCCCA AAAGANTGCC TATCCTTCCG ATGTTTCGGA TNTNTCTCGC CNTANNTNCN
661 GCCTGNNNCC TGTNTGNTGT NNNNGGACTG TGACT
```

B 2.2: Sequencing alignment of cDNA subclone **AA1#3** with **mouse peroxisomal delta 3, delta 2-enoyl-Coenzyme A isomerase (Peci)** by BLAST searching against the National Center for Biotechnology Information database

```
AA1#3      : 161 gtgtcctgcaggtcttcagattacaagtggctaaactgcacttgggcagaccaacaatga 220
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Peci: 287 gtgtcctgcaggtcttcagattacaagtggctaaactgcacttgggcagaccaacaatga 346

AA1#3      : 221 gagccagtcagcaggactttgaaaatgcattgaaccaggtgaaactcttgaagaaggacc 280
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Peci: 347 gagccagtcagcaggactttgaaaatgcattgaaccaggtgaaactcttgaagaaggacc 406

AA1#3      : 281 cgggaaacgaacgtgaagctaaagactctatgcactgtacaagcaggccacagnaaggac 340
                 |||||||||||  ||||||||  ||||||||||||||||||||||||||||||  |||||
Mouse Peci: 407 cgggaaacgaa-gtgaagct-aagactctatgcactgtacaagcaggccacag-aaggaa 463

AA1#3      : 341 cctgtaatatg 351
                 |||||||||||
Mouse Peci: 464 cctgtaatatg 474
```

B 2.3: Summary of sequence alignment of cDNA subclone **AA1#3** with **mouse Peci**

FluoroDD gel AA (AP1 + ARP2)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AA1	-	+	-	-	1400	AA1#3	1450	M13 forward (-20)	*Mus musculus* peroxisomal delta 3, delta 2-enoyl-coenzyme A isomerase (Peci) (1581 bp)	3e-85 187/191 (97%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 3.1: DNA sequence of cDNA subclone AA1#4 (AP1 & ARP2) using M13 reverse primer

```
  1 TNNCCAAGCT TGNGTACCGA GACTACNNGA TACCACTATT AAACGGACCG ACACGNGTGA
 61 CTGGAATTNC GGACTTGNTA ATAACGACTC ACTATAGGGC TTTTTTTTTT GATTTATGAA   AP1*
121 AAGTTTTATT TATCAGTACT GTGAAGAATT CTCATCATAA TTGCTACGTT AATCAAGGAA
181 AAGGCACAGA GAANCATGTG TCGTTTGAGT CCTCAGNAAA CTGGACCTCC CNAGCCCNAT
241 GCTCCCTATG AGGAGTCTAG CNTGCTGTGG TGGTCTTCAN AGCTTTGGTT TTCTGGAGAC
301 GAAGCTCNAT GATTGNGTTN CATGCAACTC CNTCTGNACN AGCCACCNTT GCCTTGCCAG
361 AAGTTAGTNC NACNTCCTTC NAGCAGTNNG ANNTGCNGTN AGCAGCATTT TTCTTTCCTC
421 CATTTTTTTC NTNGATTNAA CTCCTTNTGG NNNATTCTCC ATGGCGTTTG GGCNGGGAGC
481 TCCGCANNTG TTTTCCGGCC NGGTCCNNNN NNTCCTGTCT CAAAAAGTGC TCCTCCAGGG
541 GAAAAACNNN NCAGTTNGAA CNAAANGGCC CTTNGNNANN CNCCCAANNG CCCNTCCTTN
601 CNNTNNGNCN NTNGNTTCCA AGCCTTTTCN NTNNCCCCCN NAAAAANGNN AGNNANGCNN
661 ATCNTNCNAA NCNTGGNCCC TTTTTGCNTN GNAGCNNCAA TNNNATCTTT CCNGGNACNN
721 ANTTNNTTAA GNAAGGANCN NTNGCTTTCC CGGNGCCNCN TGGGCCCGNA GNTNN
```

B 3.2: Sequencing alignment of cDNA subclone **AA1#4** with **mouse peroxisomal delta 3, delta 2-enoyl-Coenzyme A isomerase (Peci)** by BLAST searching against the National Center for Biotechnology Information database

```
AA1#4      : 111  gatttatgaaaagttttatttatcagtactgtgaagaattctcatcataattgctacgtt 170
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Peci: 1580 gatttatgaaaagttttatttatcagtactgtgaagaattctcatcataattgctacgtt 1521

AA1#4      : 171  aatcaaggaaaaggcacagagaancatgtgtcgtttgagtcctcagnaaactggacctcc 230
                  |||||||||||||||||||||| ||||||||||||||||||    ||||||||||||||
Mouse Peci: 1520 aatcaaggaaaaggcacagagaagcatgtgtcgtttgagtcctc--gaaactggacctcc 1463

AA1#4      : 231  cnagcccnatgctccctatgaggagtctagcntgctgtggtggtcttcanagctttggtt 290
                  | ||||| |||||||||||||||||||||||| |||||||||||||||| ||||||||||
Mouse Peci: 1462 c-agccc-atgctccctatgaggagtctagc-tgctgtggtggtcttcacagctttggtt 1406

AA1#4      : 291  ttctggagacgaagctcnatgattgngtt 319
                  ||||||||||||||||| ||||||| |||
Mouse Peci: 1405 ttctggagacgaagctc-atgattgcgtt 1378
```

B 3.3: Summary of sequence alignment of cDNA subclone **AA1#4** with **mouse Peci**

FluoroDD gel AA (AP1 + ARP2)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AA1	-	+	-	-	1400	AA1#4	1450	M13 reverse	*Mus musculus* peroxisomal delta 3, delta 2-enoyl-coenzyme A isomerase (Peci) (1581 bp)	8e-80 199/209 (95%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 4.1: DNA sequence of cDNA subclone AA1#20 (AP1 & ARP2) using M13 forward (-20) primer

```
  1 TNTACGACTC ACTATAGGGC GAATTGGGCC CTCTAGAATG CATGCNTNNA CAANCGGCCG
 61 CCANTGTGAT GGATATCTGC AGAATTCGGC TTGTAATACG ACTCACTATA GGGCTTTTTT   AP1*
121 TTTTTGATTT ATGAAAAGTT TTATTTATCA GTACTGTGAA GAATTCTCAT CATAATTGCT
181 ACGTTAATCA AGGAAAAGGC ACAGAGAAGC ATGTGTCGTT TGAGTCCTCN NAACTGGACC
241 TCCCAGCCCA TGCTCCCTAT GACGANATAC TAGCTGCTGT GGTGGTCTTC ACAGCTTTGG
301 TTTTCTGGAG ACGAAGCTCA TGATTGCGTT CATGCACTCC TCTGACAGCC ACCTTGCTTG
361 CAGAGTAGTG CACTCTTCAG CGTTGACTGC GTAGAGCTTT TCTTTCTCAT TTTTTCTGAT
421 TAACTCTTTG GNAAATTCCT CATGGCGTTT GGCGGGAGCT TCGCATATAT TTTCAGCCTG
481 GTCCCGACTT CTGTCTCAAA AGTGCTCTCA GGGAAAACTT CAGTGACAAG GCCTTGAGCN
541 CAGGCCTCTC TTGCTGTCAG CTTCTTCCCA AAGAGAAGCA TCTCAGCTGC CTTTGCTGAG
601 CCCATCATCT TCNGAAACGT GTACGAAGAG CATGCNTCCN NGGCTCTGGC CGAGNTGGGC
661 TGAATGNAGT ATGAAACGTN GNCCNGTNTG ATGCCNAGAC NNNC
```

B 4.2: Sequencing alignment of cDNA subclone **AA1#20** with **mouse peroxisomal delta 3, delta 2-enoyl-Coenzyme A isomerase (Peci)** by BLAST searching against the National Center for Biotechnology Information database

```
AA1#20     : 126  gatttatgaaaagttttatttatcagtactgtgaagaattctcatcataattgctacgtt 185
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Peci: 1580 gatttatgaaaagttttatttatcagtactgtgaagaattctcatcataattgctacgtt 1521

AA1#20     : 186  aatcaaggaaaaggcacagagaagcatgtgtcgtttgagtcctcnnaactggacctccca 245
                  ||||||||||||||||||||||||||||||||||||||||||  ||||||||||||||||
Mouse Peci: 1520 aatcaaggaaaaggcacagagaagcatgtgtcgtttgagtcctcgaaactggacctccca 1461

AA1#20     : 246  gcccatgctccctatgacganatactagctgctgtggtggtcttcacagctttggttttc 305
                  |||||||||||||||||  ||    ||||||||||||||||||||||||||||||||||
Mouse Peci: 1460 gcccatgctccctatgaggagt--ctagctgctgtggtggtcttcacagctttggttttc 1403

AA1#20     : 306  tggagacgaagctcatgattgcgttcatgcactcctctgacagccaccttgcttgcagag 365
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Peci: 1402 tggagacgaagctcatgattgcgttcatgcactcctctgacagccaccttgcttgcagag 1343

AA1#20     : 366  tagtgcactcttcagcgttgactgcgtagagcttttctttctcatttttttctgattaact 425
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Peci: 1342 tagtgcactcttcagcgttgactgcgtagagcttttctttctcatttttgtctgattaact 1283

AA1#20     : 426  ctttggnaaattcctcatggcgtttggcgggagcttcgcatatattttcagcctggtccc 485
                  ||||||  ||||||  ||||||||||||||||||  |||||||||  ||||||||||||||
Mouse Peci: 1282 ctttgg-aaattc-tcatggcgtttggcgggacgttcgcatatgttttcagcctggtcca 1225

AA1#20     : 486  gacttctgtctcaaaagtgctctcagggaaaacttcagtgacaaggccttgagcncaggc 545
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||  |||||
Mouse Peci: 1224 gacttctgtctcaaaagtgctctcagggaaaacttcagtgacaaggccttgagcccaggc 1165

AA1#20     : 546  ctctcttgctgtcagcttcttcccaaagagaagcatctcagctgcctttgctgagcccat 605
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Peci: 1164 ctctcttgctgtcagcttcttcccaaagagaagcatctcagctgcctttgctgagcccat 1105

AA1#20     : 606  catcttcngaaacgtgtacgaagagcatgcntccnnggctctggccgagntgggctgaat 665
                  |||||||  ||||||||||  ||  |||||||||  |||  ||||||||||||  |  |||||||||
Mouse Peci: 1104 catcttcggaaacgtgtaagaggagcatgcttcc-gggctctggccgagtt-ggctgaat 1047

AA1#20     : 666  gnagtatgaaacgtngnccngtntgatgc 694
                  |  ||||||||||||  |  ||  ||  ||||||
Mouse Peci: 1046 ggagtatgaaacgttgccctgtctgatgc 1018
```

B 4.3: Summary of sequence alignment of cDNA subclone **AA1#20** with **mouse Peci**

FluoroDD gel AA (AP1 + ARP2)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AA1	-	+	-	-	1400	AA1#20	1700	M13 forward (-20)	*Mus musculus* peroxisomal delta 3, delta 2-enoyl-coenzyme A isomerase (Peci) (1581 bp)	0 541/569 (95%))

Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 5.1: DNA sequence of cDNA subclone AA4#1 (AP1 & ARP2) using M13 forward (-20) primer

```
  1 ANGNCCCCCC TNATACNNCC CGNNCANNTC CCGNGGNNCN NCTNANNATC GCATCGCNNC
 61 NNNAGCGGCC GCCAGCTGNT GATGGATATC CTGCNANCNA ATNCNCNCCN TGCCTAATAC
121 NNNACTCACC TATAGGGCTT TTTTTTCTGA CCNNTNCCCA TCCACNNANC NATCTATNNT   AP1*
181 NCCATCCCNA ACCAAAANGT CAGNNCNCCA CCCCTGCAGC AGCTCCACGN AGGGTACCGG
241 CACCTGNTAT TGNAATGNAG GNAAGCGCCA CACCAGTCCC NGNTCNNNGN TACCANCGNA
301 TCCTCCCCTA ACAAAGCCCC TCCCTTCCAT CGTNGGTGGN ATGGCTCAGT CCCCTTNATC
361 TGGNATCCCA CACTCTCTAA GNGAAATAAG GGCTCTGCCC GGTCCCATCA CTGGGACCCA
421 CCACCAGGAC ACTGGCAGGA ACGTACACAC TCCATCACAT GTTCNNTGCA TTATGCTCTC
481 CTCCTGCTCC CTTTNCAACA AANAGCTCCT CAATGAANNA CCCCTGNNGG NANNACANGG
541 ANNTCCCCTC CTCCCATNGG ACCCAAACTG GTNAAACANG CCCAGCNAAC CCAGGCCTNG
601 CCGANANTNN ANCAAGAAGC CCCAACTCCA NGGTTGTAGT GGGATGGGTT TTTAGCAGGG
661 NNAAGCACTG GCTTGCGGTT AGTTACCCCC AAANNNATTN TCCCAATCTT GTTANCTCAC
721 TTCTTGGCTA NNTTANNGAN TTGCCCAAAT GAGTTNCCAN AANNCTTGGG TTNTGCAAAA
781 GCCTAANTGG ANNTGTTAAT GCCCCCTGAA GTAGNGGGNA TTNNGAGGAA GGNNTGGGTA
841 NNGTTGGGGA AANGCAATCC CTNTNGGGTG GTGTNNNNGG NANNCTNNAA CATNNGGCCT
901 TATTTAAANN NTGNTCGCCT TGNGTGTTAA NNTTNTTNTC CNTTANNGGN NANTNNGGNN
961 GNNNTTNNGT GG
```

B 5.2: Sequencing alignment of cDNA subclone **AA4#1** with **mouse apolipoprotein A-V (Apoa5)** by BLAST searching against the National Center for Biotechnology Information database

```
AA4#1       : 374  tctctaagngaaataagggctctgcccggtcccatcactgggacccaccaccaggac-ac 432
                   |||||||| |||||| ||||||||||||||||||||||||| |||||||| |||||| ||
Mouse Apoa5: 1645 tctctaag-gaaataggggctctgcccggtcccatcactgg-acccaccagcaggaccac 1588

AA4#1       : 433  tggcaggaacgtacacactccatcacatgttcnntgcattatgctctcctcctgctccct 492
                   ||||||| |||||||||||||||||||||||||  ||||| |||||||||||||||||||
Mouse Apoa5: 1587 tggcagggacgtacacactccatcacatgttctctgcatcatgctctcctcctgctccct 1528

AA4#1       : 493  t 494
                   |
Mouse Apoa5: 1527 t 1527
```

B 5.3: Summary of sequence alignment of cDNA subclone **AA4#1** with **mouse Apoa5**

FluoroDD gel AA (AP1 + ARP2)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AA4	++	+	++	++	695	AA4#1	725	M13 forward (-20)	*Mus musculus* apolipoprotein A-V (Apoa5) (1819 bp)	1e-32 112/121 (92%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 6.1: DNA sequence of cDNA subclone AA4#9 (AP1 & ARP2) using M13 reverse primer

```
  1 NCNCTATTTA GGTGACCTAT AGAATACTCA AGCTATGCAT CAAGCTTGGT ACCGAGCTCG
 61 GATNCACTAG TANCGGCCGC CAGTGTGCTG GAATCGCCCT TAGCGGATAA CAATTTCACA    ARP2
121 CAGGAGCTAG CATGGTCTAT TAGGCAGAGG GTGGAGGGTC CTGCACACCA CCAAAGGTGC
181 TGCCTGTCCC AACCTACCCA GCCTCCTCAA CNTCCCCTAC TCAGGGGCAT NACANCNTCA
241 GTAGGCTTTG CAAACCCAGC TTCTGGTACT CATTTGGCAA TCCTAAGTAN CCAGAGTGAG
301 GTGACAGGNG TGGNNGATGG TGTGTGGGGT GACTGACCGC AACCCAGTGC TTGACCCGTG
361 CCGGAAACCC CATGCCCACN NACAAACCTG NNAAGTTGGG GCCTCCCCTG GTTAAAATTN
421 CTTCCCCAAG CCCCTNGGGG NNGCTTGGGC CTNGGTTAAC ACAGGTTGNG GGTNCCNATG
481 GGGAAGAAAN GGNANCCTCC CCTGGTTTTC CCCNCCNNNN GGTTNGTTNN ATTGAANAAN
541 CNNTNNTGGT TTGGGGAANN GGGGAANNCA NGGAAAGGAA AAAAACCCAT GGAATGNNNN
601 NNNAANAAAA CNNTNNGNTG GAATGTGGAA AGTTGGTGTN TTNNCCGTTN NCCCCTTGGG
661 NNCAAGNTTN GGGTTTNTCN TTGGNCNNNN GGGTGNNNGN NNTNNCNAAG NTTGGAATNT
721 TNNNGGGGNA CNNCNNGGGG GNAAAAAAAA GNCCCCCNNT TANNNNNNNC NTNTNNAAAA
781 AAANNNNNNT GNNNANTNNN NNNANATNNN NANNGANAAN NNNNAACNCN TTTNNNNNNN
841 TTNAANGNGN GNNGGGGGCT NTNNNNTANN NNAANAAATA TTNNNNNNNN NTTNNAAAAA
901 ANANAANNNN NGGTTGT
```

B 6.2: Sequencing alignment of cDNA subclone **AA4#9** with **mouse apolipoprotein A-V (Apoa5)** by BLAST searching against the National Center for Biotechnology Information database

```
AA4#9          : 126  gctagcatggtctattaggcagagggtggagggtcctgcacaccaccaaaggtgctgcct 185
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Mouse Apoa5: 1167 gctagcatggtctattaggcagagggtggagggtcctgcacaccaccaaaggtgctgc-t 1225

AA4#9          : 186  gtcccaacctacccagcctcctcaacntcccctactcaggggcatnacancntcagtagg 245
                      |||||||||||||||||||||||||||| |||||||||||||||||||| |||   |||||||||
Mouse Apoa5: 1226 gtcccaacctacccagcctcctcaac-tcccctactcaggggcattacac--tcagtagg 1282

AA4#9          : 246  ctttgcaaacccagcttctggtactcatttggcaatcctaagtanccagagtgaggtgac 305
                      |||||||||||||||||||||||||||||||||||||||||||| | ||||||||||||||
Mouse Apoa5: 1283 ctttgcaaacccagcttctggtactcatttggcaatcctaagtagcaagagtgaggtgac 1342

AA4#9          : 306  aggngtggnngatggtgtgtggggtgactgaccgcaacccagtgcttgacccgtgccgga 365
                      ||| |||| ||||||||||||||||||||||||||| |||||||||| |||||| |||
Mouse Apoa5: 1343 aggagtgggagatggtgtgtggggtgactgaccgcaagccagtgcttga-ccgtgctgga 1401

AA4#9          : 366  aaccc 370
                      |||||
Mouse Apoa5: 1402 aaccc 1406
```

B 6.3: Summary of sequence alignment of cDNA subclone **AA4#9** with **mouse Apoa5**

FluoroDD gel AA (AP1 + ARP2)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AA4	++	+	++	++	695	AA4#9	725	M13 reverse	*Mus musculus* apolipoprotein A-V (Apoa5) (1819 bp)	3e-95 231/245 (94%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 7.1: DNA sequence of cDNA subclone AA5#5 (AP1 & ARP2) using M13 forward (-20) primer

```
  1 TNNACGACTC ACTATAGGGC GAATTGGGCC CTCTAGATGC ATGCTCGAGC GGCCGCCAGT
 61 GTGATGGATA TCTGCAGAAT TCGGCTTAGC GGATAACAAT TTCACACAAG GAGCTAGCAT   ARP2*
121 GGACGGCTAA ACGAGGGTCC AACTGTCTCT TATCTTTAAT CAGCGAAATT GACCTTTNCA
181 GTGAAGAGGC TGAAATATAA TAATAAGACG AGAAGACCCT ATGGAGCTTA AATTATATAA
241 CTTATCTATT TAATTTATTA AACCTAATGG CCCAAAAACT ATAGTATAAG TTTGAAATTT
301 CGGTTGGGGT GACCTCGGAG AATAAAAAAT CCTCCGAATG ATTATAACCT AGACTTACAA
361 GTCAAAGTAA AATCAACATA TCTTATTGAC CCAGATATAT TTTGATCAAC GGACCAAGTT
421 ACCCTAGGGA TAACAGCGCA ATCCTANNTA AGGGTTCATA TCGACAATNA NGGTTNACGA
481 CCTCGATGTT GGATCANGAC ATCCCAATGG TGTANANGCT ATTAATGGGT TCGTNTTGTT
541 CANNGATTAA AGTCCTACGT GATCTGAGTT CAGACCCGAG CANTCAGGTN NGTNNCTATC
601 TATTNACGAT TTTCTCCAGT ACGAAAGGAC ANGAGANNTN NAGNCCNCCT NACNANTAAG
661 NGCNNTCANC NTANNTTANT GANNTAAATT NTAAATTNAA TATATTCGTT ANCNCCCCTA
721 NNCNGAAANN
```

B 7.2: Sequencing alignment of cDNA subclone **AA5#5** with **mouse mitochondrion** by BLAST searching against the National Center for Biotechnology Information database

```
AA5#5                 :  114  ctagcatggacggctaaacgagggtccaactgtctcttatctttaatcagcgaaattgac  173
                              ||||||||| |||||||||||||||||||||||||||||||||||||||| ||||||||||
Mouse mitochondrion:  2061  ctagcatgaacggctaaacgagggtccaactgtctcttatctttaatcagtgaaattgac  2120

AA5#5                 :  174  ctttncagtgaagaggctgaaatataataataagacgagaagaccctatggagcttaaat  233
                              |||| |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion:  2121  cttt-cagtgaagaggctgaaatataataataagacgagaagaccctatggagcttaaat  2179

AA5#5                 :  234  tatataacttatctatttaatttattaaacctaatggcccaaaaactatagtataagttt  293
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion:  2180  tatataacttatctatttaatttattaaacctaatggcccaaaaactatagtataagttt  2239

AA5#5                 :  294  gaaatttcggttggggtgacctcggagaataaaaaatcctccgaatgattataacctaga  353
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion:  2240  gaaatttcggttggggtgacctcggagaataaaaaatcctccgaatgattataacctaga  2299

AA5#5                 :  354  cttacaagtcaaagtaaaatcaacatatcttattgacccagatatattttgatcaacgga  413
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion:  2300  cttacaagtcaaagtaaaatcaacatatcttattgacccagatatattttgatcaacgga  2359

AA5#5                 :  414  ccaagttaccctagggataacagcgcaatcctanntaagggttcatatcgacaatnangg  473
                              |||||||||||||||||||||||||||||||||  |||| |||||||||||||| | ||
Mouse mitochondrion:  2360  ccaagttaccctagggataacagcgcaatcctatttaagagttcatatcgacaattaggg  2419

AA5#5                 :  474  ttnacgacctcgatgttggatcangacatcccaatggtgtanangctattaatgggttcg  533
                              || |||||||||||||||||||| |||||||||||||||||| | |||||||||| ||||||
Mouse mitochondrion:  2420  tttacgacctcgatgttggatcaggacatcccaatggtgtagaagctattaat-ggttcg  2478

AA5#5                 :  534  tnttgttcanngattaaagtcctacgtgatctgagttcagacccgagcant-caggtnng  592
                              | ||||||| |||||||||||||||||||||||||||||||||||| | ||||| | |
Mouse mitochondrion:  2479  t-ttgttcaacgattaaagtcctacgtgatctgagttcagaccggagcaatccaggtcgg  2537

AA5#5                 :  593  tnnctatctattnacgattttctccagtacgaaaggacangaga  636
                              | |||||||||| |||||||   |||||||||||||||| ||||
Mouse mitochondrion:  2538  tttctatctatttacgatttctcccagtacgaaaggacaagaga  2581
```

B 7.3: Summary of sequence alignment of cDNA subclone **AA5#5** with **mouse mitochondrion**

FluoroDD gel AA (AP1 + ARP2)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AA5	+	++	+	+	670	AA5#5	710	M13 forward (-20)	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	0 496/524 (94%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 8.1: DNA sequence of cDNA subclone AA6#1 (AP1 & ARP2) using M13 forward (-20) primer

```
  1 TTACGACTCA CTATAGGGCG AATTGGGCCC TCTAGATGCA TGCTCGNAGC GGCCGCCAGT
 61 GTGATGGATA TCTGCAGAAT TCGGCTTAGC GGATAACAAT TTCACACAAG GAGCTAGCAT   ARP2*
121 GGACGGCTAA ACGAGGGTCC AACTGTCTCT TATCTTTAAT CAGTGAAATT GACCTTTCAG
181 TGAAGAGGCT GAAATATAAT AATAAGACGA GAAGACCCTA TGGAGCTTAA ATTATATAAC
241 TTATCTATTT AATTTATTAA ACCTAATGGC CCAAAAACTA TAGTATAAGT TTGAAATTTC
301 GGTTGGGGTG ACCTCGGAGA ATAAAAAATC CCCCGAATGA TTATAACCTA GACTTACAAG
361 TCAAAGTAAA ATCAACATAT CTTATTGACC CAGATATATT TTGATCAACG GACCAAGTTA
421 CCCTAGGGAT AACAGCGCAA TCCTATTTAN GAGTTCATAT CGACAATNAG GGTTTACGAC
481 CTCGATGTTG GATCAGGACA TNCCAATGGT GTAGAAGCTA TTANTGGTTC GTTTGTNCAA
541 CGATTAAAGT CCTACGTGAT TTGAGTNCAG ANCCGAGCAN TCCAGGNTCN GTTTNTATCT
601 ATTNACGATN NCTNNCAGTA CGANNGGACA NGANANNTAN ANCCNNCNNN CAANTNNN
```

B 8.2: Sequencing alignment of cDNA subclone **AA6#1** with **mouse mitochondrion** by BLAST searching against the National Center for Biotechnology Information database

```
AA6#1                 :  114  ctagcatggacggctaaacgagggtccaactgtctcttatctttaatcagtgaaattgac  173
                              ||||||||  ||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion:  2061  ctagcatgaacggctaaacgagggtccaactgtctcttatctttaatcagtgaaattgac  2120

AA6#1                 :  174  ctttcagtgaagaggctgaaatataataataagacgagaagaccctatggagcttaaatt  233
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion:  2121  ctttcagtgaagaggctgaaatataataataagacgagaagaccctatggagcttaaatt  2180

AA6#1                 :  234  atataacttatctatttaatttattaaacctaatggcccaaaaactatagtataagtttg  293
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion:  2181  atataacttatctatttaatttattaaacctaatggcccaaaaactatagtataagtttg  2240

AA6#1                 :  294  aaatttcggttggggtgacctcggagaataaaaaatcccccgaatgattataacctagac  353
                              ||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
Mouse mitochondrion:  2241  aaatttcggttggggtgacctcggagaataaaaaatcctccgaatgattataacctagac  2300

AA6#1                 :  354  ttacaagtcaaagtaaaatcaacatatcttattgacccagatatattttgatcaacggac  413
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion:  2301  ttacaagtcaaagtaaaatcaacatatcttattgacccagatatattttgatcaacggac  2360

AA6#1                 :  414  caagttaccctagggataacagcgcaatcctatttangagttcatatcgacaatnagggt  473
                              ||||||||||||||||||||||||||||||||||||| |||||||||||||| ||||||
Mouse mitochondrion:  2361  caagttaccctagggataacagcgcaatcctatttaagagttcatatcgacaattagggt  2420

AA6#1                 :  474  ttacgacctcgatgttggatcaggacatnccaatggtgtagaagctattantggttcgtt  533
                              |||||||||||||||||||||||||||||| |||||||||||||||| ||||||||||
Mouse mitochondrion:  2421  ttacgacctcgatgttggatcaggacatcccaatggtgtagaagctattaatggttcgtt  2480

AA6#1                 :  534  tgtncaacgattaaagtcctacgtgatttgagtncaganccgagcantccaggntcngtt  593
                              ||| |||||||||||||||||||||||| ||||| |||| | ||||| |||||| || |||
Mouse mitochondrion:  2481  tgttcaacgattaaagtcctacgtgatctgagttcagaccggagcaatccagg-tcggtt  2539

AA6#1                 :  594  tntatctattnacgatnnctnncagtacgannggaca  630
                              | ||||||||  |||||  ||  ||||||||  |||||
Mouse mitochondrion:  2540  tctatctatttacgatttctcccagtacgaaaggaca  2576
```

B 8.3: Summary of sequence alignment of cDNA subclone **AA6#1** with **mouse mitochondion**

FluoroDD gel AA (AP1 + ARP2)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AA6	+	++	+	+	660	AA6#1	710	M13 forward (-20)	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	0 495/517 (95%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 9.1: DNA sequence of cDNA subclone AA6#9 (AP1 & ARP2) using M13 reverse primer

```
  1 GCCAGCTTGN GTACCGAGCT CGGATCCACT AGTTAACGGC CGCCAGTGTG CTGGAATTCG
 61 GCTTAGCGGA TAACAATTTC ACACAAGGAG TAGCATGGAC GGCTAAACGA GGGTCCAACT   ARP2*
121 GTCTCTTATC TTTAATCAGT GAAATTGACC TTTCAGTGAA GAGGCTGAAA TATAATAATA
181 AGACGAGAAG ACCCTATGGA GCTTAAATTA TATAACTTAT CTATTTAATT TATTAAACCT
241 AATGGCCCAA AAACTATAGT ATAAGTTTGA AATTTCGGTT GGGTGACCTC GGAGAATAAA
301 AAATCCTCCG AATGATTATA ACCTAGACTT ACAAGTCAAA GTAAAATCAA CATATCTTAT
361 TGACCCAGAT ATATTTTGAT CAACGGACCA AGTTACCCTA GGGATAACAG CGCAATCCTA
421 TTTAAGAGTT CATATCGACA ATNANGGTTT ACGACCTCGA TGTTGGATCA GGACATCCCA
481 ATGGTGTAGA AGCTATTAAT GGTTCGTTTG TTCAACGATT AAAGTCCTAC GTGATCTGAG
541 TTCAGACCGG AGCANTCCAG GTCGGTTTCT ATCTATTNAC GATTTCTCCC AGTACGAAAG
601 GACNAGAGAA NTAGAGCCNN CNTACNAATA NGCGCTCTCA ACTNATTTAN GAATAAAATC
661 TAAATAANNN NNTNNGTNCN CCCTCTACCN AGAGANNGTC NAAAAAAAAA AGCCNNN
```

B 9.2: Sequencing alignment of cDNA subclone **AA6#9** with **mouse mitochondrion** by BLAST searching against the National Center for Biotechnology Information database

```
AA6#9                 :  91   tagcatggacggctaaacgagggtccaactgtctcttatctttaatcagtgaaattgacc 150
                              ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2062 tagcatgaacggctaaacgagggtccaactgtctcttatctttaatcagtgaaattgacc 2121

AA6#9                 :  151  tttcagtgaagaggctgaaatataataataagacgagaagaccctatggagcttaaatta 210
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2122 tttcagtgaagaggctgaaatataataataagacgagaagaccctatggagcttaaatta 2181

AA6#9                 :  211  tataacttatctatttaatttattaaacctaatggcccaaaaactatagtataagtttga 270
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2182 tataacttatctatttaatttattaaacctaatggcccaaaaactatagtataagtttga 2241

AA6#9                 :  271  aatttcggttggg-tgacctcggagaataaaaaatcctccgaatgattataacctagact 329
                              ||||||||||||| |||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2242 aatttcggttggggtgacctcggagaataaaaaatcctccgaatgattataacctagact 2301

AA6#9                 :  330  tacaagtcaaagtaaaatcaacatatcttattgacccagatatattttgatcaacggacc 389
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2302 tacaagtcaaagtaaaatcaacatatcttattgacccagatatattttgatcaacggacc 2361

AA6#9                 :  390  aagttaccctagggataacagcgcaatcctatttaagagttcatatcgacaatnanggtt 449
                              |||||||||||||||||||||||||||||||||||||||||||||||||| | ||||
Mouse mitochondrion: 2362 aagttaccctagggataacagcgcaatcctatttaagagttcatatcgacaattagggtt 2421

AA6#9                 :  450  tacgacctcgatgttggatcaggacatcccaatggtgtagaagctattaatggttcgttt 509
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2422 tacgacctcgatgttggatcaggacatcccaatggtgtagaagctattaatggttcgttt 2481

AA6#9                 :  510  gttcaacgattaaagtcctacgtgatctgagttcagaccggagcantccaggtcggtttc 569
                              |||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Mouse mitochondrion: 2482 gttcaacgattaaagtcctacgtgatctgagttcagaccggagcaatccaggtcggtttc 2541

AA6#9                 :  570  tatctattnacgatttctcccagtacgaaaggacnagagaantagagccnncntacnaat 629
                              |||||||| |||||||||||||||||||||||| |||||| ||||||| | ||| |||
Mouse mitochondrion: 2542 tatctatttacgatttctcccagtacgaaaggacaagagaaatagagccaccttacaaat 2601

AA6#9                 :  630  angcgctctcaac-tnatttangaataaaatctaaataa 667
                              | |||||||||||| | ||||| ||||||||||||||||
Mouse mitochondrion: 2602 aagcgctctcaacttaatttatgaataaaatctaaataa 2640
```

B 9.3: Summary of sequence alignment of cDNA subclone **AA6#9** with **mouse mitochondrion**

FluoroDD gel AA (AP1 + ARP2)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AA6	+	++	+	+	660	AA6#9	710	M13 reverse	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	0 563/579 (97%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 10.1: DNA sequence of cDNA subclone AA7#3 (AP1 & ARP2) using M13 forward (-20) primer

```
  1 AATGTTACGA CTCACTATAG GGCGAATTGG GCCCTCTAGA TGCATGCTCG AGCGGCCGCC
 61 AGTGTGATGG ATATCTGCAG AATTCGGCTT AGCGGATAAC AATTTCACAC AAGGAGCTAG   ARP2*
121 CATGGACGGC TAAACGAGGG TCCAACTGTC TCTTATCTTT AATCAGTGAA ATTGACCTTT
181 CAGTGAAGAG GCTGAAATAT AATAATAAGA CGAGAAGACC CTATGGAGCT TAAATTATAT
241 AACTTATCTA TTTAATTTAT TAAACCTAAT GGCCCAAAAA CTATAGTATA AGTTTGAAAT
301 TTCGGTTGGG GTGACCTCGG AGAATAAAAA ATCCTCCGAA TGATTATAAC CTAGACTTAC
361 AAGTCAAAGT AAAATCAACA TATCTTATCG ACCCAGATAT ATCTTGATCA ACGGACCAAG
421 TTACCCTAGG GATAACAGCG CAATCCTATT TAAGAGNNCA TATCGACAAT NAGGGTANAC
481 GANNTCGATG TTGGATCANG ACATCCCCAA TGGTGTAGAA GCTATTAATG GTTCGTTTGT
541 TCAACGATAA ANGTNCTACG TGATCTGAGT TCAGANCCGA GCNATNCAGG TCGGTTTCTA
601 TCTATTNACG ATNNCTNNCA GTACGANANG ACANGAGANN NNAGCNCCNT NACNANTNNC
661 NCTNNNACNT ANTTATGAAT AAATCTAATN AANNNNTCGT NNCCNCTANC NAANGAGTNN
721 AANAAAAA
```

B 10.2: Sequencing alignment of cDNA subclone **AA7#3** with **mouse mitochondrion** by BLAST searching against the National Center for Biotechnology Information database

```
AA7#3                 : 117  ctagcatggacggctaaacgagggtccaactgtctcttatctttaatcagtgaaattgac 176
                             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2061  ctagcatgaacggctaaacgagggtccaactgtctcttatctttaatcagtgaaattgac 2120

AA7#3                 : 177  ctttcagtgaagaggctgaaatataataataagacgagaagaccctatggagcttaaatt 236
                             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2121  ctttcagtgaagaggctgaaatataataataagacgagaagaccctatggagcttaaatt 2180

AA7#3                 : 237  atataacttatctatttaatttattaaacctaatggcccaaaaactatagtataagtttg 296
                             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2181  atataacttatctatttaatttattaaacctaatggcccaaaaactatagtataagtttg 2240

AA7#3                 : 297  aaatttcggttggggtgacctcggagaataaaaaatcctccgaatgattataacctagac 356
                             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2241  aaatttcggttggggtgacctcggagaataaaaaatcctccgaatgattataacctagac 2300

AA7#3                 : 357  ttacaagtcaaagtaaaatcaacatatcttatcgacccagatatatcttgatcaacggac 416
                             |||||||||||||||||||||||||||||||||||| ||||||||||||| ||||||||||||
Mouse mitochondrion: 2301  ttacaagtcaaagtaaaatcaacatatcttattgacccagatatattttgatcaacggac 2360

AA7#3                 : 417  caagttaccctagggataacagcgcaatcctatttaagagnncatatcgacaatnagggt 476
                             |||||||||||||||||||||||||||||||||||||||| |||||||||||| ||||||
Mouse mitochondrion: 2361  caagttaccctagggataacagcgcaatcctatttaagagttcatatcgacaattagggt 2420

AA7#3                 : 477  anacgannтcgatgttggatcangacatccccaatggtgtagaagctattaatggttcgt 536
                             ||||  |||||||||||||||| |||||| ||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2421  ttacgacctcgatgttggatcaggacat-cccaatggtgtagaagctattaatggttcgt 2479

AA7#3                 : 537  ttgttcaacgataaangtnctacgtgatctgagttcaganccgagcnatncaggtcggtt 596
                             |||||||||||| || || |||||||||||||||||| | |||| || ||||||||||||
Mouse mitochondrion: 2480  ttgttcaacgattaaagtcctacgtgatctgagttcagaccggagcaatccaggtcggtt 2539

AA7#3                 : 597  tctatctattnacgatnnctnncagtacganangacangaga 638
                             |||||||||| |||||  ||  |||||||| | |||| ||||
Mouse mitochondrion: 2540  tctatctatttacgatttctcccagtacgaaaggacaagaga 2581
```

B 10.3: Summary of sequence alignment of cDNA subclone **AA7#3** with **mouse mitochondrion**

FluoroDD gel AA (AP1 + ARP2)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AA7	+	++	+	+	640	AA7#3	710	M13 forward (-20)	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	0 495/522 (94%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 11.1: DNA sequence of cDNA subclone AA7#5 (AP1 & ARP2) using M13 reverse primer

```
  1 GCCAAGCTTG NGTACCGAGC TCGGATCCAC TAGTAACGGC CGCCAGTGTG CTGGAATTCG
 61 GCTNGTAATA CGACTCACTA TAGGGCTTTT TTTTTTTGAC CTTCTCTAGG TTAGAGGGTG   AP1
121 TACGTATATA TTTTATTTAG ATTTTATTCA TAAATTAAGT TGAGAGCGCT TATTTGTAAG
181 GTGGCTCTAT TTCTCTTGTC CTTTCGTACT GGGAGAAATC GTAAATAGAT AGAAACCGAC
241 CTGGATTGCT CCGGTCTGAA CTCAGATCAC GTAGGACTTT AATCGTTGAA CAAACGAACC
301 ATTAATAGCT TCTACACCAT TGGGATGTCC TGATCCAACA TCGAGGTCGT AAACCCTAAT
361 TGTCGATATG AACTCTTAAA TAGGATTGCG CTGTTATCCC TAGGGTAACT TGGTCCGTTG
421 ATCAAAATAT ATCTGGGTCA ATAAGATATG TNGATTTTAC TTTGACTNGT AAGTCTAGGT
481 NATAATCATT CGGANGATNT TTATTCTNCG AGGTCNNCCN ANNGAAATTT CAACTTATAC
541 TATAGTTTTN GGNCATANGT TTATAATAAA TAGATAGTAT ATANTTANCN CATAGGTCTC
601 TGTCTATATA ATTCGCTCTC CTGAAGTCAT CACTATNANA TGAACGTGAN NGTTAGCGTA
661 GN
```

B 11.2: Sequencing alignment of cDNA subclone **AA7#5** with **mouse mitochondrion** by BLAST searching against the National Center for Biotechnology Information database

```
AA7#5                : 99   accttctctaggttagagggtgtacgtatatattttatttagattttattcataaattaa 158
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2674 accttctctaggttagagggtgtacgtatatattttatttagattttattcataaattaa 2615

AA7#5                : 159  gttgagagcgcttatttgtaaggtggctctatttctcttgtcctttcgtactgggagaaa 218
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2614 gttgagagcgcttatttgtaaggtggctctatttctcttgtcctttcgtactgggagaaa 2555

AA7#5                : 219  tcgtaaatagatagaaaccgacctggattgctccggtctgaactcagatcacgtaggact 278
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2554 tcgtaaatagatagaaaccgacctggattgctccggtctgaactcagatcacgtaggact 2495

AA7#5                : 279  ttaatcgttgaacaaacgaaccattaatagcttctacaccattgggatgtcctgatccaa 338
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2494 ttaatcgttgaacaaacgaaccattaatagcttctacaccattgggatgtcctgatccaa 2435

AA7#5                : 339  catcgaggtcgtaaaccctaattgtcgatatgaactcttaaataggattgcgctgttatc 398
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2434 catcgaggtcgtaaaccctaattgtcgatatgaactcttaaataggattgcgctgttatc 2375

AA7#5                : 399  cctagggtaacttggtccgttgatcaaaatatatctgggtcaataagatatgtngatttt 458
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Mouse mitochondrion: 2374 cctagggtaacttggtccgttgatcaaaatatatctgggtcaataagatatgttgatttt 2315

AA7#5                : 459  actttgactngtaagtctaggtnataatcattcgganga-tntttattctncgaggtc-n 516
                            ||||||||| |||||||||||| |||||||||||||| || | |||||||| |||||||
Mouse mitochondrion: 2314 actttgacttgtaagtctaggttataatcattcggaggatttttattctccgaggtcac 2255

AA7#5                : 517  nccnanngaaatttc-aacttatactatagtttt 549
                              || |  |||||||| ||||||||||||||||||
Mouse mitochondrion: 2254 cccaaccgaaatttcaaacttatactatagtttt 2221
```

B 11.3: Summary of sequence alignment of cDNA subclone **AA7#5** with **mouse mitochondrion**

FluoroDD gel AA (AP1 + ARP2)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AA7	+	++	+	+	640	AA7#5	710	M13 reverse	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	0 440/454 (96%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 12.1: DNA sequence of cDNA subclone AA10#1 (AP1 & ARP2) using M13 forward (-20) primer

```
  1 TATACGACTC ACTATAGGGC GAATTGGGCC CTCTAGAATG CATGCTCGAG CGGCCGCCAG
 61 TGTGATGGAT ATCTGCAGAA TTCGGCTTAG CGGATAACAA TTTCACACAA GGAGCTAGCA   ARP2*
121 TGGTGAAGAA GGGGACCATG ATGATTGGCT ACCAGCCCCA TGGGACCCGG GCCAACTTCT
181 TCCGAATGGT GGTGGCCAAC CCCATACTGG CCCAGGCCGA TATANGATTT CCTTCTNNGG
241 CGAGCTGGAG CTCCTGGGCC AGGAACCTGT GAGCTGCTTC TGCTCTCTGC CCCACCCAAA
301 GCTCTGCGTA GTCTCCTGGG TTCTCAAAAT CGACCTTTCT AGGAAACAGT GGCCTTGNAC
361 TGTGTGTGCT CCCACACACT CACTCTCCCA GCTAAGTATT GGCTGTCAGG AAGGTGTGCT
421 AAACACACAC ACCTACTGTA TGTTCTTAAA CGAAAATATG CTTANATNAA AAGTNGGGCA
481 ACAANGGNGC ACACACCCNT TTANCTACCC AGCATTGGGG NGGGACNNAA AGCAGCCCAG
541 TNNTGAANGC CAGCCTGAAC TACAGAGCTN GGCTACACAG AAAAACTGTC TCNNANANNN
601 AAAGCCCNNN TNNTNNATTN NNNTTNCCGC CNGATTNCCN GCCCNCNNGG CNNGGCNGNT
661 NCNTANGTNG GATNCNGAGC CCTCGGNTAN NCCAAGCCNT NNGGCNGTTA NNTCTTTGGN
721 NTNNTNNNCG N
```

B 12.2: Sequencing alignment of cDNA subclone **AA10#1** with **mouse cysteine sulfinic acid decarboxylase (Csad)** by BLAST searching against the National Center for Biotechnology Information database

```
AA10#1     : 118  gcatggtgaagaaggggaccatgatgattggctaccagccccatgggacccgggccaact 177
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Csad: 1500 gcatggtgaagaaggggaccatgatgattggctaccagccccatgggacccgggccaact 1559

AA10#1     : 178  tcttccgaatggtggtggccaaccccatactggcccaggccgatatangatttccttctn 237
                  ||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Mouse Csad: 1560 tcttccgaatggtggtggccaaccccatactggcccaggccgatata-gatttccttct- 1617

AA10#1     : 238  nggcgagctggagctcctgggccaggaacctgtgagctgcttctgctctctgccccaccc 297
                  |||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Mouse Csad: 1618 gggcgagctggagctcctgggccagg-acctgtgagctgcttctgctctctgccccaccc 1676

AA10#1     : 298  aaagctctgcgtagtctcctgggttctcaaaatcgacctttctaggaaacagtggccttg 357
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Csad: 1677 -aagctctgcgtagtctcctgggttctcaaaatcgacctttctaggaaacagtggccttg 1735

AA10#1     : 358  nactgtgtgtgctcccacacactcactctcccagctaagtattggctgtcaggaaggtgt 417
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Csad: 1736 -actgtgtgtgctcccacacactcactctcccagctaagtattggctgtcaggaaggtgt 1794

AA10#1     : 418  gctaaacacac 428
                  ||||||||||
Mouse Csad: 1795 -ctaaacacac 1804
```

B 12.3: Summary of sequence alignment of cDNA subclone **AA10#1** with **mouse Csad**

FluoroDD gel AA (AP1 + ARP2)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AA10	+	++	+	+	500	AA10#1	550	M13 forward (-20)	*Mus musculus* cysteine sulfinic acid decarboxylase (Csad) (2265 bp)	e-143 304/311 (97%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 13.1: DNA sequence of cDNA subclone AA10#1 (AP1 & ARP2) using M13 reverse primer

```
  1 TCAGCCAGCT TGNGTACCGA GACTCGGATN CACTATTAAC GGCCGCCAGT GTGCTGGAAT
 61 TCGGCTGTAA TACGACTCAC TATAGGGACT TTTTTTTTTT TGAGANCAGT TTCNNTGTGT   AP1
121 AGACCCAGAC NNTGTAGTCC AGGACTGGNC CTCAAACTGG ACTGCTTCTG TNACCCCCAA
181 TGCTGGTAGT AAAGGTGTGT GCCCCTGTGN CCCANNTTAA TAAAAGACAT ATTTCGTAAG
241 ANNATAACTG TAGTGTGTTT AAGAACACCC TTCCTGNNAG CCAATACTTA GACTGGGAGA
301 GTGAGTGTGT GGGANNNNNA NCAACAGTCA AGGCCNACTG TTTCCCTANG AAAAGGTCCG
361 ATTTTGANGA NACCCAGGAG AACTANCGNC AGAGCTTGGG TGGGGNCAGA NGANGCAGAA
421 AGCCGCNCCA CAGGTCCCTG GNCCCAGGAG NCTCCNGNCT CGCCCCAGAA ANNAAAATCT
481 ATANTCGGCC CCGCCGCCAC GTAATGGGNG NTCGGCCCCC CCCCCCCTNC CCGAANNAAC
541 GTNNGGNCCC GGGGGTCCCC ANTNNGGGCC TNGGTANGCC CANTTTATTN CNTNGGGTNN
601 CCCCCTNTCT TTCCCNCNNT GGCTAGCCTC CCNTNNGNTN GNTGANNNTT TNGNTTANTT
661 CCCGGNTNAN NGNCCCGAAA TTNTTTGGGC NNNNNTNNTN NNCTTTCCCN CCNNNGGGNG
721 GGGGGAGGGT TTTNGNNGNC NNT
```

B 13.2: Sequencing alignment of cDNA subclone **AA10#1** with **mouse cysteine sulfinic acid decarboxylase (Csad)** by BLAST searching against the National Center for Biotechnology Information database

```
AA10#1     : 133  tgtagtccaggactggncctcaaactggactgcttctgtnacccccaatgctggtagtaa 192
                  |||||||||||| |||| |||||||||||| |||||||||| ||||||||||||||||||
Mouse Csad: 1917 tgtagtccagg-ctgg-cctcaaactgg-ctgcttctgtc-cccccaatgctggtagtaa 1862

AA10#1     : 193  aggtgtgtgcccctgtgncccannttaataaaagacatatttcgtaagannataactgta 252
                  |||||||||||||||| ||||  |||||||||| |||||||||||||  || ||||||
Mouse Csad: 1861 aggtgtgtgcccctgtg-cccaacttaataaaag-catatttcgtaagaacat-actgta 1805

AA10#1     : 253  gtgtgtttaagaacacccttcctgnnagccaatacttagactgggagagtgagtgtgtgg 312
                  |||||||||  ||| ||||||||  |||||||||||||| ||||||||||||||||||||
Mouse Csad: 1804 gtgtgttta--gaca-ccttcctgacagccaatacttag-ctgggagagtgagtgtgtgg 1749

AA10#1     : 313  ga 314
                  ||
Mouse Csad: 1748 ga 1747
```

B 13.3: Summary of sequence alignment of cDNA subclone **AA10#1** with **mouse Csad**

FluoroDD gel AA (AP1 + ARP2)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AA10	+	++	+	+	500	AA10#1	550	M13 reverse	*Mus musculus* cysteine sulfinic acid decarboxylase (Csad) (2265 bp)	3e-32 163/182 (89%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 14.1: DNA sequence of cDNA subclone AA12#4 (AP1 & ARP2) using M13 forward (-20) primer

```
  1 CCCACCNCAC TTNAGGGCGA ATTGGGCCCT CTAGATGCAT GCTCGAGCGG CCGCCAGTGT
 61 GATGGATATC TGCAGAATTC GCCCTTAGCG GATAACAATT TCACACAGGA GCTAGCATGG   ARP2
121 CAGCGGCGTT CCGCAGAGGC TGCAGGGTCC TGAGAAGTGT TTCTCATTTT GAGTGTCGAA
181 CACAACACTC GAAAGCGGCT CACAAGCAGG AGCCCGGATT AGGGTTTAGT TTTGAGTTGA
241 CGGAACAGCA GAAAGAGTTT CAAGCAACTG CCCGCAAGTT TGCCAGAGAG GAGATTATCC
301 CCGTCGCCCC GGAATATGAC AAAAGCGGGG AGTACCCGTT CCCTCTCATC AAAAGAGCCT
361 GGGAACTCGG CTTGATCAAC GCGCACATTC CGGAAAGTTG CGGTGGTCTT GGCCTGGGAA
421 CGTTCGATGC TTGTTTAATT ACCGAAGAGT TGGCGTATGG GTGTACAGGG GTGCAAACTG
481 CTATTGAAGC AAATTCTTTG GGGCAAATGC CTGTGATTCT TGCTGGAAAT GATCAAAAAA
541 AAGCCCTATA GTGAGTCGTA TTACAAGGGC GAATTCCAGC ACACTGGCGG CCCGTTACTA
601 GTGGATNCGA GCTCGGTACC AAGCTTGATG CATAGCTTGA GTATTCTATA GTGTCACCTA
661 AATAGCTNGG CGTAATCATG GTCATAGCTG TNNCCTGTGT GAAATGGTTA TCCGCTCACN
721 NTTNCACACA AATACNAGCN GGNANCATAA GTGTAAGNCN GGGNTGCTAA TGAGTGAGCT
781 AA
```

B 14.2: Sequencing alignment of cDNA subclone **AA12#4** with **mouse acetyl-coenzyme A dehydrogenase, medium chain (MCAD)** by BLAST searching against the National Center for Biotechnology Information database

```
AA12#4     : 116 catggcagcggcgttccgcagaggctgcagggtcctgagaagtgtttctcattttgagtg 175
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse MCAD: 3   catggcagcggcgttccgcagaggctgcagggtcctgagaagtgtttctcattttgagtg 62

AA12#4     : 176 tcgaacacaacactcgaaagcggctcacaagcaggagcccggattagggtttagttttga 235
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse MCAD: 63  tcgaacacaacactcgaaagcggctcacaagcaggagcccggattagggtttagttttga 122

AA12#4     : 236 gttgacggaacagcagaaagagtttcaagcaactgcccgcaagtttgccagagaggagat 295
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse MCAD: 123 gttgacggaacagcagaaagagtttcaagcaactgcccgcaagtttgccagagaggagat 182

AA12#4     : 296 tatccccgtcgccccggaatatgacaaaagcggggagtacccgttccctctcatcaaaag 355
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse MCAD: 183 tatccccgtcgccccggaatatgacaaaagcggggagtacccgttccctctcatcaaaag 242

AA12#4     : 356 agcctgggaactcggcttgatcaacgcgcacattccggaaagttgcggtggtcttggcct 415
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse MCAD: 243 agcctgggaactcggcttgatcaacgcgcacattccggaaagttgcggtggtcttggcct 302

AA12#4     : 416 gggaacgttcgatgcttgtttaattaccgaagagttggcgtatgggtgtacaggggtgca 475
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse MCAD: 303 gggaacgttcgatgcttgtttaattaccgaagagttggcgtatgggtgtacaggggtgca 362

AA12#4     : 476 aactgctattgaagcaaattctttggggcaaatgcctgtgattcttgctggaaatgatc 534
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse MCAD: 363 aactgctattgaagcaaattctttggggcaaatgcctgtgattcttgctggaaatgatc 421
```

B 14.3: Summary of sequence alignment of cDNA subclone **AA12#4** with **mouse MCAD**

FluoroDD gel AA (AP1 + ARP2)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AA12	+	++	+	+	470	AA12#4	500	M13 forward (-20)	*Mus musculus* acetyl-coenzyme A dehydrogenase, medium chain (MCAD) (1857 bp)	0 419/419 (100%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 15.1: DNA sequence of cDNA subclone AA12#4 (AP1 & ARP2) using M13 reverse primer

```
  1 AGACTATTAG GTGACNCTAT AGAATACTCA AGNCTATGCA TCAAGCTTGG TACCGAGCTC
 61 GGATCCACTA GTAACGGCCG CCAGTGTGCT GGAATTCGCC CTTGTAATAC GACTCACTAT   AP1*
121 AGGGCTTTTT TTTGATCATT TCCAGCAAGA ATCACAGGCA TTTGCCCCAA AGAATTTGCT
181 TCAATAGNCA GTTTGCNCCC CTGTACACCC ATACGACCAA CTCTTCGGTA ATTAAANCAA
241 GNCATCNGAA ACGTNNCCCA GGGNCCAAGA ANCCANCCGC CAAACTTTTN CCGGGAAATG
301 GTGNCGNCGN TTGNNNNAAG NCCGANGTNC CCCAGGGCTC TTTTGATGAA GANGGAAACN
361 GGGTACTCCC CNCTTTTNNT ATATNNCGGG GCGACNGGNN TAATNCTCCT CNCTGNNAAA
421 CTNNCGGGNN CNNTGCTTGG AAACTCTTTC CTGGCTGTTC CCGGTTAANC CNNAAAAACT
481 AAAAACCCTN AAATNCCGGG GGCTNCCTGC CTTTGGNNNA GNCCGCTTTT CCGAGGTGGT
541 TGNTGGTTNC GACACCNNAA AAAATGANNA AAACNCTTTC NNAGGGAACC CTGNAGGNCT
601 CCTGGGCGAA NCGNCCGNCN GNNCANTGCC AACCNNCCCT GGTGNNNAAA AATTGNTTAA
661 TTNCCGCTAA NGGGGGCGAA TTNNTNGNNN NANTNATTNC ACTNCNNACN NNGGNCNNCC
721 GCGNTTCNNA CNTTNNNNNN NANGANNNNC CCCNNTTTNN CCCTANTNNN TGGANNTNGT
781 AANTTAAANN AAATTTAACN NNNGNNNCGG
```

B 15.2: Sequencing alignment of cDNA subclone **AA12#4** with **mouse acetyl-coenzyme A dehydrogenase, medium chain (MCAD)** by BLAST searching against the National Center for Biotechnology Information database

```
AA12#4     : 134 gatcatttccagcaagaatcacaggcatttgccccaaagaatttgcttcaatagncagtt 193
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Mouse MCAD: 421 gatcatttccagcaagaatcacaggcatttgccccaaagaatttgcttcaatag-cagtt 363

AA12#4     : 194 tgcnccccctgtacacccatacgaccaactcttcggtaattaaa 236
                 ||| |||||||||||||||||| ||||||||||||||||||||
Mouse MCAD: 362 tgcacccctgtacacccatacg-ccaactcttcggtaattaaa 321
```

B 15.3: Summary of sequence alignment of cDNA subclone **AA12#4** with **mouse MCAD**

FluoroDD gel AA (AP1 + ARP2)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AA12	+	++	+	+	470	AA12#4	500	M13 reverse	*Mus musculus* acetyl-coenzyme A dehydrogenase, medium chain (MCAD) (1857 bp)	5e-38 100/103 (97%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 16.1: DNA sequence of cDNA subclone AB7#2 (AP3 & ARP3) using M13 forward (-20) primer

```
  1 TGGGCCCTCT AGATGCATGC TCGAGCGGCC GCCAGTGTGA TGGATATCTG CAGAATTCGC
 61 CCTTAGCGGA TAACAATTTC ACACAGGAGA CCATTGCAAA CCTGCTAAAC CATTGCCTAA   ARP3
121 GGAAATGGAA GACTTTGTTC AGAGCTCTGG AGAGCATGGT GTGGTGGTGT TTTCTCTGGG
181 GTCAATGGTC AGTAATATGA CAGAAGAAAA GGCCAATGCA ATTGCATGGG CTCTTGCCCA
241 GATTCCACAA AAGGTTCTTT GGAGATTTGA CGGCAAAACT CCAGCCACCT TAGGACCCAA
301 TACCAGAATC TACAAGTGGC TTCCCCAAAA TGATCTTCTT GGTCATCCAA AAACCAAAGC
361 CTTTATAACT CATGGTGGCG CGAATGGACT CTACGAGGCG ATCCATCATG GAATCCCTAT
421 GATTGGCATT CCTTTGTTTG GAGAACAACA TGATAACATT GCTCACATGG TGGCCAAAGG
481 AGCAGCTGTT ACATTGAATA TCAGAACGAT GTCCAGGTCA GATTTGCTCA ATGCACTGGA
541 GGAAGTCATA GACAATCCTT TCTATAAAGA GAATGCTATG TGGTTGTCAA CCATTCACCA
601 TGACCAGCCT ATGAAGCCCC TGGACAGAGC AGTCTTCTGG ATNGAGTTTG TCATGCGCCA
661 CAAAGGNGCC AAGCATCTGA GACCACTNGC ATACNACCTC ACCTGGTANC AGTACCACTC
721 TCTGNATGTG ATNGNATCCT GNNGCNTTNG TANNTNNTGT ACCCNCATGT AAATCTTTTG
781 TGGTAACCAN NNT
```

B 16.2: Sequencing alignment of cDNA subclone **AB7#2** with **mouse UDP-glucuronosyltransferase 2 family, member 5 (UGT2b5)** by BLAST searching against the National Center for Biotechnology Information database

```
AB7#2     : 91   ccattgcaaacctgctaaaccattgcctaaggaaatggaagactttgttcagagctctgg 150
Mouse UGT: 868   ccattgcaaacctgctaaacccttgcctaaggatatggaagaatttgtccagagctctgg 927

AB7#2     : 151  agagcatggtgtggtggtgttttctctggggtcaatggtcagtaatatgacagaagaaaa 210
Mouse UGT: 928   agaccatggtgtggtggtcttttctcttgggtcaatggttagtaacatgacagaagaaaa 987

AB7#2     : 211  ggccaatgcaattgcatgggctcttgcccagattccacaaaaggttctttggagatttga 270
Mouse UGT: 988   agccaacgcaattgcatgggcccttgcccagattccacaaaaggttctttggaaatttga 1047

AB7#2     : 271  cggcaaaactccagccaccttaggacccaataccagaatctacaagtggcttccccaaaa 330
Mouse UGT: 1048  tggcaaaaccccagcaactttaggacacaataccagagtgtacaagtggctcccccagaa 1107

AB7#2     : 331  tgatcttcttggtcatccaaaaaccaaagcctttataactcatggtggcgcgaatggact 390
Mouse UGT: 1108  tgacctccttggtcatccaaaaaccaaagcctttgtaactcatggtggggccaatggtgt 1167

AB7#2     : 391  ctacgaggcgatccatcatggaatccctatgattggcattcctttgtttggagaacaaca 450
Mouse UGT: 1168  ctacgaggcgatctatcatggaatccctatgattggcattcctttgtttggagaacagca 1227

AB7#2     : 451  tgataacattgctcacatggtggccaaaggagcagctgttacattgaatatcagaacgat 510
Mouse UGT: 1228  tgataacattgcccatatggtggccaaaggagcagctgttgcattgaatatcagaacaat 1287

AB7#2     : 511  gtccaggtcagatttgctcaatgcactggaggaagtcatagacaatcctttctataaaga 570
Mouse UGT: 1288  gtcaaagtcagatgtgctcaatgcactggaggaggtcatagaaaatcctttctataaaaa 1347

AB7#2     : 571  gaatgctatgtggttgtcaaccattcaccatgaccagcctatgaagcccctggacagagc 630
Mouse UGT: 1348  gaatgctatgtggttgtcaaccattcaccatgaccagcctatgaaaccccctggacagggc 1407

AB7#2     : 631  agtcttctggatngagtttgtcatgcgccacaaaggngccaagcatctgagaccactngc 690
Mouse UGT: 1408  tgtcttctggattgagtttgttatgcgccacaaaagagccaagcacctgagaccacttgg 1467

AB7#2     : 691  atacnacctcacctggtancagtaccactctctgnatgtgat 732
Mouse UGT: 1468  acataaccttacctggtaccagtaccactctctggatgtgat 1509
```

B 16.3: Summary of sequence alignment of cDNA subclone **AB7#2** with **mouse UGT2b5**

FluoroDD gel AB (AP3 + ARP3)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AB7	+	++	+	+	850	AB7#2	900	M13 forward (-20)	Mouse UDP-glucuronosyltransferase 2 family, member 5 (UGT2b5) (1648 bp)	0 585/642 (91%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 17.1: DNA sequence of cDNA subclone AB7#8 (AP3 & ARP3) using M13 reverse primer

```
  1 TAGAATACTC AAGCTATGCA TCAAGCTTGG TACCGAGCTC GGATCCACTA GTAACGGCCG
 61 CCAGTGTGCT GGAATTCGCC CTTGTAATAC GACTCACTAT AGGGCTTTTT TTTTTTGGTG    AP3*
121 GTAAAATTTT ATTCATGTAA TGCATTATCA ATGAGTTCTA CTCATTCTTC ATTTTCTTTT
181 CTTTCTTTAC AAAGAATCGG TAAACAAACA AGAAGCACTT TACAATGAGG GCTACAATGA
241 ATGTCACAAA AGCAAGCAGG AATCCAATCA CATCCAGAGA GTGGTACTGG TACCAGGTGA
301 GGTTGTATGC AAGTGGTCTC AGATGCTTGG CCCCTTTGTG GCGCATGACA AACTCAATCC
361 AGAAGACTGC TCTGTCCAGG GGCTTCATAG GCTGGTCATG GTGAATGGTT GACAACCACA
421 TAGCATTCTC TTTATAGAAA GGATTGTCTA TGACTTCCTC CAGTGCATTG AGCAAATCTG
481 ACCTTGACAT CGTTCTGATA TTCAATGTAA CAGCTGCTCC TTTGGCCACC ATGTGAGCAA
541 TGTTATCATG TTGTTCTCCA AACAAAGGAA TGCCAATCAT AGGGATNCCA TGATGGATCG
601 CCTCGTAGAG TCCATTCGCG CCACCATGAG TNATAAAGGC TTTGGTTNTT GGATGACCAA
661 GAAGATCATT TTGGGGANGC ACTNGTANGT TCTGGTATTG GGTCTAAGGT GGCTGGAGTN
721 NNCCGTCAAA TCTCCAAGAA CCTTNGTGAA A
```

B 17.2: Sequencing alignment of cDNA subclone **AB7#8** with **mouse UDP-glucuronosyltransferase 2 family, member 5 (UGT2b5)** by BLAST searching against the National Center for Biotechnology Information database

```
AB7#8     : 143  cattatcaatgagttctactcattcttcattttcttttctttctttacaaagaatcggta 202
                 |||| ||| |||| ||||||||||||||||||| ||||||||||||||||||||||||||
Mouse UGT: 1633 cattgtcagtgagctctactcattcttcattttatttttctttctttacaaagaatcggta 1574

AB7#8     : 203  aacaaacaagaagcactttacaatgagggctacaatgaatgtcacaaaagcaagcaggaa 262
                 ||  ||||||| |||||||||| | ||| ||| | ||  || |||| |||  || |||||
Mouse UGT: 1573 aatgaacaagaggcactttacactaaggactatagtggttgccacacaagagagtaggaa 1514

AB7#8     : 263  tccaatcacatccagagagtggtactggtaccaggtgaggttgtatgcaagtggtctcag 322
                 |||||||||||||||||||||||||||||||||||| ||||| | | ||||||||||||||
Mouse UGT: 1513 tccaatcacatccagagagtggtactggtaccaggtaaggttatgtccaagtggtctcag 1454

AB7#8     : 323  atgcttggcccctttgtggcgcatgacaaactcaatccagaagactgctctgtccagggg 382
                 |||||||| | |||||||||||| ||||||||||||||||||| || ||||||||||||
Mouse UGT: 1453 gtgcttggctcttttgtggcgcataacaaactcaatccagaagacagccctgtccagggg 1394

AB7#8     : 383  cttcataggctggtcatggtgaatggttgacaaccacatagcattctctttatagaaagg 442
                 ||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Mouse UGT: 1393 tttcataggctggtcatggtgaatggttgacaaccacatagcattcttttatagaaagg 1334

AB7#8     : 443  attgtctatgacttcctccagtgcattgagcaaatctgaccttgacatcgttctgatatt 502
                 ||| |||||||| |||||||||||||||||| ||||||| |||||||| ||||||||||||
Mouse UGT: 1333 attttctatgacctcctccagtgcattgagcacatctgactttgacattgttctgatatt 1274

AB7#8     : 503  caatgtaacagctgctcctttggccaccatgtgagcaatgttatcatgttgttctccaaa 562
                 |||||| |||||||||||||||||||||||||| || |||||||||||| ||||||||||
Mouse UGT: 1273 caatgcaacagctgctcctttggccaccatatgggcaatgttatcatgctgttctccaaa 1214

AB7#8     : 563  caaaggaatgccaatcatagggatnccatgatggatcgcctcgtagagtccattcgcgcc 622
                 |||||||||||||||||||||||||| ||||||| |||||||||||| ||||| || ||
Mouse UGT: 1213 caaaggaatgccaatcatagggattccatgatagatcgcctcgtagacaccattggcccc 1154

AB7#8     : 623  accatgagtnataaaggctttggttnttggatgaccaagaagatcattttgggg 676
                 |||||||||| | |||||||||||||| |||||||||||| || ||||| |||||
Mouse UGT: 1153 accatgagttacaaaggctttggtttttggatgaccaaggaggtcattctgggg 1100
```

B 17.3: Summary of sequence alignment of cDNA subclone **AB7#8** with **mouse UGT2b5**

FluoroDD gel AB (AP3 + ARP3)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AB7	+	++	+	+	850	AB7#8	900	M13 reverse	Mouse UDP-glucuronosyltransferase 2 family, member 5 (UGT2b5) (1648 bp)	0 482/534 (90%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 18.1: DNA sequence of cDNA subclone AB17#16 (AP3 & ARP3) using M13 reverse primer

```
  1 CCAGACTATT TAGGTGACCT ATAGAATACT CNANNTATGC NTCAAGCTTG GTACCGAGCT
 61 CGGATCCACT AGTAACGGCC GCCAGTGTGC TGGAATTCGC CCTTAGCGGA TAACAATTTC   ARP3
121 ACACAGGAGA CCATTGCACA TAGGACTTAT TCTTCTTACA TGACAAAAAA TTGCTCCCCT
181 ATCAATTTTA ATTCAAATTT ACCCGCTACT CAACTCTACT ATCATTTTAA TACTAGCAAT
241 TACTTCTATT TTCATAGGGG CATGAGGAGG ACTTAACCAA ACACAAATAC GAAAAATTAT
301 AGCCTATTCA TCAATTGCCC ACATAGGATG AATATTAGCA ATTCTTNCCT TACAACCCAT
361 CCCTCACTCT ACTCAACCTC ATAATCTATA TTATTCTTAC AGCCCCTATA TTCATAGCAC
421 TTATACTAAA TAACTCTATA ACCATCAACT CAATCTCACT TCTATGAAAT AAAACTCCAG
481 CAATACTAAC TATAATCTCA CTGATATTAC TATCNCTANG AGGCCTNNCA CCACTANCAN
541 GATTCTNACC CAAATGANTT ATCATCACAG AACTTATAAA AANCACTGTC TANTATAGCA
601 NACTCATAGC ANTATAGCTC TACTAACCTT CTTATCCGCC TATNANNANT ATACAATTCA
661 CACATNNCAA A
```

B 18.2: Sequencing alignment of cDNA subclone **AB17#16** with **mouse mitochondrion** by BLAST searching against the National Center for Biotechnology Information database

```
AB17#16                 : 135  tgcacataggacttattcttcttacatgacaaaaaattgctcccctatcaattttaattc 194
                                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4284 tgcacataggacttattcttcttacatgacaaaaaattgctcccctatcaattttaattc 4343

AB17#16                 : 195  aaatttacccgctactcaactctactatcattttaatactagcaattacttctattttca 254
                                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4344 aaatttacccgctactcaactctactatcattttaatactagcaattacttctattttca 4403

AB17#16                 : 255  taggggcatgaggaggacttaaccaaacacaaatacgaaaaattatagcctattcatcaa 314
                                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4404 taggggcatgaggaggacttaaccaaacacaaatacgaaaaattatagcctattcatcaa 4463

AB17#16                 : 315  ttgcccacataggatgaatattagcaattcttnccttacaacccatccctcactctactc 374
                                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4464 ttgcccacataggatgaatattagcaattctt-ccttacaacccatccctcactctactc 4522

AB17#16                 : 375  aacctcataatctatattattcttacagcccctatattcatagcacttatactaaataac 434
                                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4523 aacctcataatctatattattcttacagcccctatattcatagcacttatactaaataac 4582

AB17#16                 : 435  tctataaccatcaactcaatctcacttctatgaaataaaactccagcaatactaactata 494
                                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4583 tctataaccatcaactcaatctcacttctatgaaataaaactccagcaatactaactata 4642

AB17#16                 : 495  atctcactgatattactatcnctangaggcctnncaccactancangattctnacccaaa 554
                                ||||||||||||||||||| ||| ||||||| |||||||| || |||||| ||| |||
Mouse mitochondrion: 4643 atctcactgatattactatccctaggaggccttccaccactaacaggattcttaccaaaa 4702

AB17#16                 : 555  tganttatcatcacagaacttataaaaanc-actgtct-antatagc-anactcatagca 611
                                ||| |||||||||||||||||||||||| | ||||||| | |||||| | ||||||||||
Mouse mitochondrion: 4703 tgaattatcatcacagaacttataaaaaacaactgtctaattatagcaacactcatagca 4762

AB17#16                 : 612  nt-atagctctactaa 626
                                | ||||||||||||||
Mouse mitochondrion: 4763 ataatagctctactaa 4778
```

B 18.3: Summary of sequence alignment of cDNA subclone **AB17#16** with **mouse mitochondrion**

FluoroDD gel AB (AP3 + ARP3)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AB17	+	++	+	+	620	AB17#16	650	M13 reverse	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	0 478/496 (96%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 19.1: DNA sequence of cDNA subclone AB18#4 (AP3 & ARP3) using M13 forward (-20) primer

```
  1 TATACGACTC ACTATAGGGC GAATTGGGCC CTCTAGATGC ATGCTCGNAG CGGCCGCCAG
 61 TGTGATGGAT ATCTGCAGAA TTCGGCTTAG CGGATAACAA TTTCACACAG GAGACCATTG   ARP3
121 CACATAGGAC TTATTCTTCT TACATGACAA AAAATTGCTC CCCTATCAAT TTTAATTCAA
181 ATTTACCCGC TACTCAACTC TACTATCATT TTAATACTAG CAATTACTTC TATTTTCATA
241 GGGGCATGAG GAGGACTTAA CCAAACACAA ATACGAAAAA TTATAGCCTA TTCATCAATT
301 GCCCACATAG GATGAATATT AGCAATTCTT CCTTACAACC CATCCCTCAC TCTACTCAAC
361 CTCATAATCT ATATTATTCT TACAGCCCCT ATATTCATAG CACTTATACT AAATAACTCT
421 ATAACCATCA ACTCAATCTC ACTTCTATGA AATAAAACTC CAGCAATACT AACTATAATC
481 TCACTGATAT TACTATCCCT ANGANGCCTC CACCACTAAC AGGATNCTNA CCAANATGAA
541 TTATCATCAC AGAACTNATA AAAAACNACT GCCTAATTAT AGCANACTCA TAGCCATANT
601 AGCTCTCTAA NCTATTCTTT ATACTCNNNT ANTTATTCAC TNNNCTANNN NATNNCCACC
661 CACAATACCC AAAANAANN
```

B 19.2: Sequencing alignment of cDNA subclone **AB18#4** with **mouse mitochondrion** by BLAST searching against the National Center for Biotechnology Information database

```
AB18#4              : 119  tgcacataggacttattcttcttacatgacaaaaaattgctcccctatcaattttaattc 178
                           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4284  tgcacataggacttattcttcttacatgacaaaaaattgctcccctatcaattttaattc 4343

AB18#4              : 179  aaatttacccgctactcaactctactatcattttaatactagcaattacttctattttca 238
                           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4344  aaatttacccgctactcaactctactatcattttaatactagcaattacttctattttca 4403

AB18#4              : 239  taggggcatgaggaggacttaaccaaacacaaatacgaaaaattatagcctattcatcaa 298
                           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4404  taggggcatgaggaggacttaaccaaacacaaatacgaaaaattatagcctattcatcaa 4463

AB18#4              : 299  ttgcccacataggatgaatattagcaattcttccttacaacccatccctcactctactca 358
                           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4464  ttgcccacataggatgaatattagcaattcttccttacaacccatccctcactctactca 4523

AB18#4              : 359  acctcataatctatattattcttacagcccctatattcatagcacttatactaaataact 418
                           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4524  acctcataatctatattattcttacagcccctatattcatagcacttatactaaataact 4583

AB18#4              : 419  ctataaccatcaactcaatctcacttctatgaaataaaactccagcaatactaactataa 478
                           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4584  ctataaccatcaactcaatctcacttctatgaaataaaactccagcaatactaactataa 4643

AB18#4              : 479  tctcactgatattactatccctangangcc-tccaccactaacaggatnctnaccaanat 537
                           |||||||||||||||||||||||| || ||| |||||||||||||||| || ||||| ||
Mouse mitochondrion: 4644  tctcactgatattactatccctaggaggccttccaccactaacaggattcttaccaaaat 4703

AB18#4              : 538  gaattatcatcacagaactnataaaaaacnactgcctaattatagc-anactcatagcca 596
                           |||||||||||||||||||| ||||||||| |||| |||||||||| | |||||||| |
Mouse mitochondrion: 4704  gaattatcatcacagaacttataaaaaacaactgtctaattatagcaacactcatagcaa 4763

AB18#4              : 597  tantagctct 606
                           || |||||||
Mouse mitochondrion: 4764  taatagctct 4773
```

B 19.3: Summary of sequence alignment of cDNA subclone **AB18#4** with **mouse mitochondrion**

FluoroDD gel AB (AP3 + ARP3)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AB18	+	++	+	+	595	AB18#4	655	M13 forward (-20)	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	0 477/490 (97%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 20.1: DNA sequence of cDNA subclone AB18#4 (AP3 & ARP3) using M13 reverse primer

```
  1 NGCCAAGCTT GNGTACCGAG CTCGGATCCA CTAGTAACGG CCGCCAGTGT GCTGGAATTC
 61 GGCTNGTAAT ACGACTCACT ATAGGGCTTT TTTTTTTTTG GGTTATTGTT GGTTGGAAAT   AP3
121 ATTGTTAGTG AAGTGGAATA AATTAGGCGA GTATAAAAGA ATAGGTTTAG TAGAGCTATT
181 ATTGCTATGA GTGTTGCTAT AATTAGGCAG TTGTTTTTTA TAAGTTCTGT GATGATAATT
241 CATTTTGGTA AGAATCCTGT TAGTGGTGGA AGGCCTCCTA GGGATAGTAA TATCAGTGAG
301 ATTATAGTTA GTATTGCTGG AGTTTTATTT CATAGAAGTG AGATTGAGTT GATGGTTATA
361 GAGTTATTTA GTATAAGTGC TATGAATATA GGGGCTGTAA GAATAATATA GATTATGAGG
421 TTGAGTAGAG TGAGGGATGG GTTGTAAGGA AGAATNGCTA ATATTCATCC TATGTGGGCA
481 ATTGATGAAT ANGCTATAAT NTNTCGTATN TGTGTTTGGT NAAGTCCTCC TCATGCCCCT
541 ATGAAAATAG AAGTAATTGC TAGTATNAAA ATGATAGTAG AGTTGAGTAG CNGGTAAATT
601 TGAATTAAAT TGATANNGGA GCANTTTTTG TCATGTAGAG ATAGTCTATG TGCATGTCTC
661 GTGTAATGTA TCGTAGCGAT CGCNAT
```

B 20.2: Sequencing alignment of cDNA subclone **AB18#4** with **mouse mitochondrion** by BLAST searching against the National Center for Biotechnology Information database

```
AB18#4                : 102  gttattgttggttggaaatattgttagtgaagtggaataaattaggcgagtataaaagaa 161
                             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4843 gttattgttggttggaaatattgttagtgaagtggaataaattaggcgagtataaaagaa 4784

AB18#4                : 162  taggtttagtagagctattattgctatgagtgttgctataattaggcagttgttttttat 221
                             ||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Mouse mitochondrion: 4783 taggtttagtagagctattattgctatgagtgttgctataattagacagttgttttttat 4724

AB18#4                : 222  aagttctgtgatgataattcattttggtaagaatcctgttagtggtggaaggcctcctag 281
                             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4723 aagttctgtgatgataattcattttggtaagaatcctgttagtggtggaaggcctcctag 4664

AB18#4                : 282  ggatagtaatatcagtgagattatagttagtattgctggagttttatttcatagaagtga 341
                             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4663 ggatagtaatatcagtgagattatagttagtattgctggagttttatttcatagaagtga 4604

AB18#4                : 342  gattgagttgatggttatagagttatttagtataagtgctatgaatatagggqctgtaag 401
                             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4603 gattgagttgatggttatagagttatttagtataagtgctatgaatatagggqctgtaag 4544

AB18#4                : 402  aataatatagattatgaggttgagtagagtgagggatgggttgtaaggaagaatngctaa 461
                             ||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Mouse mitochondrion: 4543 aataatatagattatgaggttgagtagagtgagggatgggttgtaaggaagaattgctaa 4484

AB18#4                : 462  tattcatcctatgtgggcaattgatgaatangctataatntntcgtatntgtgtttggtn 521
                             |||||||||||||||||||||||||||||||| ||||||| | |||||| ||||||||||
Mouse mitochondrion: 4483 tattcatcctatgtgggcaattgatgaataggctataatttttcgtatttgtgtttggtt 4424

AB18#4                : 522  aagtcctcctcatgcccctatgaaaatagaagtaattgctagtatnaaaatgatagtaga 581
                             ||||||||||||||||||||||||||||||||||||||||||||||| |||||||||||||
Mouse mitochondrion: 4423 aagtcctcctcatgcccctatgaaaatagaagtaattgctagtattaaaatgatagtaga 4364

AB18#4                : 582  gttgagtagcnggtaaatttgaatt-aaattgatannggagc-anttttgtcatgta 637
                             |||||||||| |||||||||||||| |||||||||  |||||| | ||||||||||||
Mouse mitochondrion: 4363 gttgagtagcgggtaaatttgaattaaaattgataggggagcaatttttgtcatgta 4306
```

B 20.3: Summary of sequence alignment of cDNA subclone **AB18#4** with **mouse mitochondrion**

FluoroDD gel AB (AP3 + ARP3)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AB18	+	++	+	+	595	AB18#4	655	M13 reverse	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	0 524/538 (97%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 21.1: DNA sequence of cDNA subclone AB19#2 (AP3 & ARP3) using M13 forward (-20) primer

```
  1 TGGGCCCTCT AGATGCATGC TCGAGCGGCC GCCAGTGTGA TGGATATCTG CAGAATTCGC
 61 CCTTGTATAC GACTCACTAT AGGGCTTTTT TTTTTTGGG TGGAATAAAT TAGGCGAGTA   AP3
121 TAAAAGAATA GGTTTAGTAG AGCTATTATT GCTATGAGTG TTGCTATAAT TAGACAGTTG
181 TTTTTTATAA GTTCTGTGAT GATAATTCAT TTTGGTAAGA ATCCTGTTAG TGGTGGAAGG
241 CCTCCTAGGG ATAGTAATAT CAGTGAGATT ATAGTTAGTA TTGCTGGAGT TTTATTTCAT
301 AGAAGTGAGA TTGAGTTGAT GGTTATAGAG TTATTTAGTA TAAGTGCTAT GAATATAGGG
361 GCTGTAAGAA TAATATAGAT TATGAGGTTG AGTAGAGTGA GGGATGGGTT GTAAGGAAGA
421 ATNGCTAATA TTCATCCTAT GTGGGCAATT GATGAATAGG CTATAATTTT TCGTATNTGT
481 GTNNNGGTTA AGTCCTCCTC ATGCCCCTAT GAAAATAGAA GTAATTGCTA GTATTAAAAT
541 GATAGTAGAG TTGAGTAGCG GGTAAATTTG AATTAAAATT GATANGGGAG CAATTTTTTG
601 TCATGTAAGA AGAATAAGTC CTATGTGCAA TGGTCTNCTG TGTGAANTNG TNATCCGCTA
661 AGGGCGAATN CCAGCACACT GGCGNCCGTA ACTAGTGGAT CGAGCTCNGT ACCANGCCTG
721 ATGCATAGCN TGANNNTTCT ATAGTGTNAN CTAATA
```

B 21.2: Sequencing alignment of cDNA subclone **AB19#2** with **mouse mitochondrion** by BLAST searching against the National Center for Biotechnology Information database

```
AB19#2                : 100  gtggaataaattaggcgagtataaaagaataggtttagtagagctattattgctatgagt 159
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4812  gtggaataaattaggcgagtataaaagaataggtttagtagagctattattgctatgagt 4753

AB19#2                : 160  gttgctataattagacagttgtttttttataagttctgtgatgataattcattttggtaag 219
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4752  gttgctataattagacagttgtttttttataagttctgtgatgataattcattttggtaag 4693

AB19#2                : 220  aatcctgttagtggtggaaggcctcctagggatagtaatatcagtgagattatagttagt 279
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4692  aatcctgttagtggtggaaggcctcctagggatagtaatatcagtgagattatagttagt 4633

AB19#2                : 280  attgctggagttttatttcatagaagtgagattgagttgatggttatagagttatttagt 339
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4632  attgctggagttttatttcatagaagtgagattgagttgatggttatagagttatttagt 4573

AB19#2                : 340  ataagtgctatgaatatagggggctgtaagaataatatagattatgaggttgagtagagtg 399
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4572  ataagtgctatgaatatagggggctgtaagaataatatagattatgaggttgagtagagtg 4513

AB19#2                : 400  agggatgggttgtaaggaagaatngctaatattcatcctatgtgggcaattgatgaatag 459
                              |||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4512  agggatgggttgtaaggaagaattgctaatattcatcctatgtgggcaattgatgaatag 4453

AB19#2                : 460  gctataatttttcgtatntgtgtnnnggttaagtcctcctcatgcccctatgaaaataga 519
                              |||||||||||||||||| |||||  ||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4452  gctataatttttcgtatttgtgt-ttggttaagtcctcctcatgcccctatgaaaataga 4394

AB19#2                : 520  agtaattgctagtattaaaatgatagtagagttgagtagcgggtaaatttgaattaaaat 579
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4393  agtaattgctagtattaaaatgatagtagagttgagtagcgggtaaatttgaattaaaat 4334

AB19#2                : 580  tgatangggagcaatttttttgtcatgtaagaagaataagtcctatgtgca 629
                              ||||| ||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4333  tgataggggagcaatttttttgtcatgtaagaagaataagtcctatgtgca 4284
```

B 21.3: Summary of sequence alignment of cDNA subclone **AB19#2** with **mouse mitochondrion**

FluoroDD gel AB (AP3 + ARP3)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AB19	-	+	-	-	575	AB19#2	620	M13 forward (-20)	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	0 523/530 (98%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 22.1: DNA sequence of cDNA subclone AB19#10 (AP3 & ARP3) using M13 reverse primer

```
  1 TAGANTACTC AAGCTATGCA TCAAGCTTGG TACCGAGCTC GGATCCACTA GTAACGGCCG
 61 CCAGTGTGCT GGAATTCGCC CTTAGCGGAT AACAATTTCA CACAGGAGAC CATTGCACAT   ARP3
121 AGGACTTATT CTTCTTACAT GACAAAAAAT TGCTCCCCTA TCAATTTTAA TTCAAATTTA
181 CCCGCTACTC AACTCTACTA TCATTTTAAT ACTAGCAATT ACTTCTATTT TCATAGGGGC
241 ATGAGGAGGA CTTAACCAAA CACAAATACG AAAAATTATA GCCTATTCAT CAATTGCCCA
301 CATAGGATGA ATATTAGCAA TTCTTCCTTA CAACCCATCC CTCACTCTAC TCAACCTCAT
361 AATCTATATT ATTCTTACAG CCCCTATATT CATAGCACTT ATACTAAATA ACTCTATAAC
421 CATCAACTCA ATCTCACTTC TATGGAATAA AACTCCAGCA ATACTAACTA TAATCTCACT
481 GATATTACTA TCCCTAGGAG GCCTTCCACC ACTAACAGGA TTCTTACCAA AATGAATTAT
541 CATCACAGAA CTTATAAAAA ACAACTGTCT AATTATAGCA ACACTCATAG CAATAATAGC
601 TCTACTAAAC CTATTCTTTN ATACTCGCCC CAAAAAAAAA AAGCCCTATA GTGAGTCGTA
661 TNACAAGGGC GAATTCTGCA GATATCATCA CACTGNCGGG CGCTCGAGCA TGCATNNANA
721 GGCCNCATTT CNCCNTATAG TGAGNTTGTA TNACNATNCA CNGCCNGNTG NTNAACANCG
781 GTGTGACNNG NNAACNNNGC C
```

B 22.2: Sequencing alignment of cDNA subclone **AB19#10** with **mouse mitochondrion** by BLAST searching against the National Center for Biotechnology Information database

```
AB19#10           : 114  tgcacataggacttattcttcttacatgacaaaaaattgctcccctatcaattttaattc 173
                         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4284 tgcacataggacttattcttcttacatgacaaaaaattgctcccctatcaattttaattc 4343

AB19#10           : 174  aaatttacccgctactcaactctactatcattttaatactagcaattacttctattttca 233
                         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4344 aaatttacccgctactcaactctactatcattttaatactagcaattacttctattttca 4403

AB19#10           : 234  taggggcatgaggaggacttaaccaaacacaaatacgaaaaattatagcctattcatcaa 293
                         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4404 taggggcatgaggaggacttaaccaaacacaaatacgaaaaattatagcctattcatcaa 4463

AB19#10           : 294  ttgcccacataggatgaatattagcaattcttccttacaacccatccctcactctactca 353
                         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4464 ttgcccacataggatgaatattagcaattcttccttacaacccatccctcactctactca 4523

AB19#10           : 354  acctcataatctatattattcttacagcccctatattcatagcacttatactaaataact 413
                         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4524 acctcataatctatattattcttacagcccctatattcatagcacttatactaaataact 4583

AB19#10           : 414  ctataaccatcaactcaatctcacttctatggaataaaactccagcaatactaactataa 473
                         |||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4584 ctataaccatcaactcaatctcacttctatgaaataaaactccagcaatactaactataa 4643

AB19#10           : 474  tctcactgatattactatccctaggaggccttccaccactaacaggattcttaccaaaat 533
                         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4644 tctcactgatattactatccctaggaggccttccaccactaacaggattcttaccaaaat 4703

AB19#10           : 534  gaattatcatcacagaacttataaaaaacaactgtctaattatagcaacactcatagcaa 593
                         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 4704 gaattatcatcacagaacttataaaaaacaactgtctaattatagcaacactcatagcaa 4763

AB19#10           : 594  taatagctctactaaacctattctttnatactcgcc 629
                         |||||||||||||||||||||||||| |||||||||
Mouse mitochondrion: 4764 taatagctctactaaacctattcttttatactcgcc 4799
```

B 22.3: Summary of sequence alignment of cDNA subclone **AB19#10** with **mouse mitochondrion**

FluoroDD gel AB (AP3 + ARP3)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AB19	-	+	-	-	575	AB19#10	610	M13 reverse	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	0 513/516 (99%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 23.1: DNA sequence of cDNA subclone AB22#9 (AP3 & ARP3) using M13 forward (-20) primer

```
  1 CANTTGGGCC CTCTAGATGC NTGCTCGAGC GGCCGCCAGT GTGATGGATA TCTGCAGAAT
 61 TCGCCCTTGT AATACGACTC ACTATAGGGC TTTTTTTTTC TTGGCACTAC CAGAGTCAGG   AP3*
121 TTTATTAGAG AAGAGGGGCT TGGGGGAACT GGAGGGCTGA GGGGCCCTGC CCAGAGCAGC
181 AGAGCACAGC ACATGGCCCT CTGCCCCAGC TCCCTCATGG CTGTCAGCCC CAACTGTAGA
241 AATAGATTTT CTGCCAGGAG GGCAAGTAGT CCATGAGCGG CCTTGCCACC AGACCCACGC
301 CAGGGATGTG GTCTGTAAGC AGCTGGAGCA GGAAGAGGAT CTTGGCTTCA GAGAAGCGGT
361 NATAAGAACC GGTGAGCGTA GCAGGTANTA TAGCAGCAGG ATTGTGCGAC CGCCTCAGTT
421 CTAGCCGCTC CCTCCGGGTC AAGTTCTTCC TGTNCNTCAG GAANACTCAG GCTATTCNTA
481 TTTACCACAC CANANNGTTN NCCAGGGTGT NNNACGATCG CTGACCCCCT AAGCNANCTG
541 ACCNAGTGCA CAGGGGCCNN GGCGATGTAC AAAANANTTG CCATGGTTTT CNTGTTGTNA
601 AAATTGTATC NGCTTANGGG NNANTNNGGG CNGCNTNNTN TGATCCACNN NNNNNTNNTN
661 ANTTTTNNT
```

B 23.2: Sequencing alignment of cDNA subclone **AB22#9** with **mouse peroxisome biogenesis factor 16 (Pex16)** by BLAST searching against the National Center for Biotechnology Information database

```
AB22#9      : 105  cactaccagagtcaggtttattagagaagaggggcttgggggaactggagggctgagggg 164
                   ||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Mouse Pex16: 1196 cactaccagagtcaggtttattagagaagaggggcctgggggaactggagggctgagggg 1137

AB22#9      : 165  ccctgcccagagcagcagagcacagcacatggccctctgccccagctccctcatggctgt 224
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Pex16: 1136 ccctgcccagagcagcagagcacagcacatggccctctgccccagctccctcatggctgt 1077

AB22#9      : 225  cagccccaactgtagaaatagattttctgccaggagggcaagtagtccatgagcggcctt 284
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Pex16: 1076 cagccccaactgtagaaatagattttctgccaggagggcaagtagtccatgagcggcctt 1017

AB22#9      : 285  gccaccagacccacgccagggatgtggtctgtaagcagctggagcaggaagaggatcttg 344
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Pex16: 1016 gccaccagacccacgccagggatgtggtctgtaagcagctggagcaggaagaggatcttg 957

AB22#9      : 345  gcttcagagaagcggtnataagaaccggtgagcgtagcaggtantatagcagcaggattg 404
                   |||||||||||||||| || |||| |||||||||||||||||| ||||||||||||||||
Mouse Pex16: 956  gcttcagagaagcggtcat-agaa-cggtgagcgtagcaggtagtatagcagcaggattg 899

AB22#9      : 405  tgcgaccgcctcagttctagccgctccctccgggtcaagttcttcctgtncntcaggaan 464
                   ||||| |||||||||||||||||||||| |||||||||||||||||    ||||| |
Mouse Pex16: 898  tgcga-cgcctcagttctagccgctccctctgggtcaagttcttcctgtcactcagg-ag 841

AB22#9      : 465  actcaggctattcntatttaccacaccanannngttnnccagggtgtnnnacgatcgctga 524
                   |||||||||| || ||| |||||||||| | | | |||||||||   ||||||||||
Mouse Pex16: 840  actcaggctagtcatatctaccacaccagacag-tagccagggtgt-ccacgatcgctga 783

AB22#9      : 525  cccccctaagc 534
                   |||| |||||
Mouse Pex16: 782  ccccataagc 773
```

B 23.3: Summary of sequence alignment of cDNA subclone **AB22#9** with **mouse Pex16**

FluoroDD gel AB (AP3 + ARP3)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AB22	+	++	+	+	530	AB22#9	580	M13 forward (-20)	*Mus musculus* peroxisome biogenesis factor 16 (Pex16) (1221 bp)	e-178 405/430 (94%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 24.1: DNA sequence of cDNA subclone AB22#9 (AP3 & ARP3) using M13 reverse primer

```
  1 AATACTCAAG CTATGCATCA AGCTTGGTAC CGAGCTCGGA TCCACTAGTA ACGGCCGCCA
 61 GTGTGCTGGA ATTCGCCCTT AGCGGATAAC AATTTCACAC AGGAGACCAT TGCAGAGTCT   ARP3
121 TTGTACATCG CCCGGCCCCT GCTGCACTTG CTCAGCCTGG GCTTATGGGG TCAGCGATCG
181 TGGACACCCT GGCTACTGTC TGGTGTGGTA GATATGACTA GCCTGAGTCT CCTGAGTGAC
241 AGGAAGAACT TGACCCGGAG GGAGCGGCTA GAACTGAGGC GTCGCACAAT CCTGCTGCTA
301 TACTACCTGC TACGCTCACC GTTCTATGAC CGCTTCTCTG AAGCCAAGAT CCTCTTCCTG
361 CTCCAGCTGC TTACAGACCA CATCCCTGGC GTGGGTCTGG TGGCAAGGCC GCTCATGGAC
421 TACTTGCCCT CCTGGCAGAA AATCTATTTC TACAGTTGGG GCTGACAGCC ATGAGGGAGC
481 TGGGGCAGAG GGCCATGTGC TGTGCTCTGC TGCTCTGGGC AGGGCCCCTC AGCCCTCCAG
541 TTCCCCCAAG CCNCTCTTCT CTAATAAACC CTGACTCTGG TAGTGCCAAG ANAAAAAAAG
601 CCCTATAGTG AGTNTTATNA CAAGGNCGAA TTCTGCAAAT ATCCATCACA CTGGCGGCCG
661 CTCNANCCTG CATTTAGAGG GCCCNATCCC CCTATAGTGA GTNNTATNAC NNNNNCTGGC
721 CGTTTTTTAC ATCTTNTTGA CTGGAAANCN CTGGGTTA
```

B 24.2: Sequencing alignment of cDNA subclone **AB22#9** with **mouse peroxisome biogenesis factor 16 (Pex16)** by BLAST searching against the National Center for Biotechnology Information database

```
AB22#9      : 100  caggagaccattgcagagtctttgtacatcgcccggcccctgctgcacttgctcagcctg 159
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Pex16: 712   caggagaccattgcagagtctttgtacatcgcccggcccctgctgcacttgctcagcctg 771

AB22#9      : 160  ggcttatggggtcagcgatcgtggacaccctggctactgtctggtgtggtagatatgact 219
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Pex16: 772   ggcttatggggtcagcgatcgtggacaccctggctactgtctggtgtggtagatatgact 831

AB22#9      : 220  agcctgagtctcctgagtgacaggaagaacttgacccggagggagcggctagaactgagg 279
                   |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Mouse Pex16: 832   agcctgagtctcctgagtgacaggaagaacttgacccagagggagcggctagaactgagg 891

AB22#9      : 280  cgtcgcacaatcctgctgctatactacctgctacgctcaccgttctatgaccgcttctct 339
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Pex16: 892   cgtcgcacaatcctgctgctatactacctgctacgctcaccgttctatgaccgcttctct 951

AB22#9      : 340  gaagccaagatcctcttcctgctccagctgcttacagaccacatccctggcgtgggtctg 399
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Pex16: 952   gaagccaagatcctcttcctgctccagctgcttacagaccacatccctggcgtgggtctg 1011

AB22#9      : 400  gtggcaaggccgctcatggactacttgccctcctggcagaaaatctatttctacagttgg 459
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Pex16: 1012  gtggcaaggccgctcatggactacttgccctcctggcagaaaatctatttctacagttgg 1071

AB22#9      : 460  ggctgacagccatgagggagctggggcagagggccatgtgctgtgctctgctgctctggg 519
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Pex16: 1072  ggctgacagccatgagggagctggggcagagggccatgtgctgtgctctgctgctctggg 1131

AB22#9      : 520  cagggcccctcagccctccagttcccccaagccnctcttctctaataaaccctgactctg 579
                   |||||||||||||||||||||||||||||||| ||| ||||||||||||||| ||||||||||
Mouse Pex16: 1132  cagggcccctcagccctccagttcccccaggcccctcttctctaataaa-cctgactctg 1190

AB22#9      : 580  gtagtg 585
                   ||||||
Mouse Pex16: 1191  gtagtg 1196
```

B 24.3: Summary of sequence alignment of cDNA subclone **AB22#9** with **mouse Pex16**

FluoroDD gel AB (AP3 + ARP3)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AB22	+	++	+	+	530	AB22#9	580	M13 reverse	*Mus musculus* peroxisome biogenesis factor 16 (Pex16) (1221 bp)	0 482/486 (99%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 25.1: DNA sequence of cDNA subclone AB24#9 (AP3 & ARP3) using M13 forward (-20) primer

```
  1 CCNCAACTAT AGGGCAATTG GGCCCTCTAG ATGCNTGCTC GAGCGGCCGC CAGTGTGATG
 61 GATATCTGCA GAATTCGCCC TTAGCGGATA ACAATTTCAC ACAGGAGACC ATTGCACTGA   ARP3
121 CAGAGGAAGA TATATTTTAG AGGGAGACAC TTGTACCTTT CTCTCCCTTC AGTTATTAGA
181 CTCTTGGGAC AATGGACATC ATGAATTAAA ACGTTCTTAG AAATCACATG CTGGGAGAAA
241 ATTAACACTA AAATCTGGTA CCAGCCAGAG GAAGGAACTT GACTCAAAAT AAGAGATTTT
301 TAGATATTTC TGTCTGTCTC ATAGTTAAAA TTAATGTTCT CCTGCTTTCT GGCATATGCC
361 TCATCTTTTC TATGAAGTAG TAATACTGAT ACAGAAAGGT AGAGAGAAAT GAATAGTTTT
421 TGCTACTTTG GGCCAAACTG TGAAAAAATC CATTTTATTT CACCAAAAAA AAAAAAGCCC
481 TATAGTGAGT CGTATTACAA GGGCGAATNC CAGCACACTG GCGGCCGTTA CTAGTGGATC
541 CGAGCTCGGT ACCAAGCTNG ATGCATAGCT TGAGTATTCT ATAGTGTCAC CTAAATAGCT
601 TGGCGTAATC ATGGTAATAG CTGTTTCCTG TGTGAAATNG TNATCCGCTC ACNATTCCAC
661 ACAACATACG ANCCNGANCC NTAANGTGTA AAGCNTGGGG TGCCTAATGA GTGANCTACT
721 CACATANNTG CGTGCNCTAC TGCCGNNTCA GTNGGNAACT GTNTCNNCNG CNTATANNNC
781 CACCCGNGGA AGCGGTGNNT TGGCCCTTCC TTNNNTAAAT GNGCCGGTTT TGGGGGAG
```

B 25.2: Sequencing alignment of cDNA subclone **AB24#9** with **mouse Cyp4a14** by BLAST searching against the National Center for Biotechnology Information database

```
AB24#9          : 108  accattgcactgacagaggaagatatattttagagggagacacttgtacctttctctccc 167
                       ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14:  1823  accattgcactgacagaggaagatatattttagagggagacacttgtacctttctctccc 1882

AB24#9          : 168  ttcagttattagactcttgggacaatggacatcatgaattaaaacgttcttagaaatcac 227
                       ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14:  1883  ttcagttattagactcttgggacaatggacatcatgaattaaaacgttcttagaaatcac 1942

AB24#9          : 228  atgctgggagaaaattaacactaaaatctggtaccagccagaggaaggaacttgactcaa 287
                       ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14:  1943  atgctgggagaaaattaacactaaaatctggtaccagccagaggaaggaacttgactcaa 2002

AB24#9          : 288  aataagagatttttagatatttctgtctgtctcatagttaaaattaatgttctcctgctt 347
                       ||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||
Mouse Cyp4a14:  2003  aataagagatttttagatatttctgtctgtctcatagttaaaattaatgttttcctgctt 2062

AB24#9          : 348  tctggcatatgcctcatcttttctatgaagtagtaatactgatacagaaaggtagagaga 407
                       ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14:  2063  tctggcatatgcctcatcttttctatgaagtagtaatactgatacagaaaggtagagaga 2122

AB24#9          : 408  aatgaatagtttttgctactttgggccaaactgtgaaaaaatccattttatttca 462
                       |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14:  2123  aatgaatagtttttgctactttgggccaaactgtgaaaaaatccattttatttca 2177
```

B 25.3: Summary of sequence alignment of cDNA subclone **AB24#9** with **mouse Cyp4a14**

FluoroDD gel AB (AP3 + ARP3)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AB24	-	+	-	-	420	AB24#9	460	M13 forward (-20)	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 14 (Cyp4a14) (2547 bp)	0 354/355 (99%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 26.1: DNA sequence of cDNA subclone AB24#9 (AP3 & ARP3) using M13 reverse primer

```
  1 AANNNTATTT AGGTGACACT ATAGAATACT CAAGCTATGC ATCAAGCTTG GTACCGAGCT
 61 CGGATCCACT AGTAACGGCC GCCAGTGTGC TGGAATTCGC CCTTGTAATA CGACTCACTA   AP3
121 TAGGGCTTTT TTTTTTTTGG TGAAATAAAA TGGATTTTTT CACAGTTTGG CCCAAAGTAG
181 CAAAAACTAT TCATTTCTCT CTACCTTTCT GTATCAGTAT TACTACTTCA TAGAAAAGAT
241 GAGGCATATG CCAGAAAGCA GGAGAACATT AATTTTAACT ATGAGACAGA CAGAAATATC
301 TAAAAATCTC TTATTTTGAG TCAAGTTCCT TCCTCTGGCT GGTACCAGAT TTTAGTGTTA
361 ATTTTCTCCC AGCATGTGAT TTCTAAGAAC GTTTTAATTC ATGATGTCCC ATTGTCCCAA
421 GANTTATTAA TAACTGAAGG GAGAGAAAGG TACAAGTGTC TCCCCCTCTA AAATATATCC
481 TTCCCTCCTG TCAGTGCNAA TGGGTCTCCT GTGTGNAAAT TGTTATCCGC TAAGGGCGAA
541 TTNTGCAGAT ATCCAATNNA AACAAAATAA AACAAACAAG CCNTGCNNTT TAGANNGGGC
601 CCAATTTGGC CCNTATAGTT GANGTNGTAT TACAAATNNA NNTGGGCCGT TGTTTTNANN
661 ANNTNNNTGA ATGGNAAAAC CCTGGCNGTN ACNNNNNTNA ATGNCNNTNG NNGCNNNTTC
721 CCTTTTNNCN NCTGGGNTNN TANNAAAAAG NCCNCCCGAA TGCCTTTCCA ACANTTGGCC
781 NCCTGAATGN NNNTNGNCCN CCNNNTTNNN GCCNNTNNNN NNGNNGGTTG NTTNNNNNTN
841 NNNT
```

B 26.2: Sequencing alignment of cDNA subclone **AB24#9** with **mouse Cyp4a14** by BLAST searching against the National Center for Biotechnology Information database

```
AB24#9          : 141  tgaaataaaatggatttttttcacagtttggcccaaagtagcaaaaactattcatttctct 200
                       ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14: 2177 tgaaataaaatggatttttttcacagtttggcccaaagtagcaaaaactattcatttctct 2118

AB24#9          : 201  ctacctttctgtatcagtattactacttcatagaaaagatgaggcatatgccagaaagca 260
                       ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14: 2117 ctacctttctgtatcagtattactacttcatagaaaagatgaggcatatgccagaaagca 2058

AB24#9          : 261  ggagaacattaattttaactatgagacagacagaaatatctaaaaatctcttattttgag 320
                       ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14: 2057 ggaaaacattaattttaactatgagacagacagaaatatctaaaaatctcttattttgag 1998

AB24#9          : 321  tcaagttccttcctctggctggtaccagattttagtgttaattttctcccagcatgtgat 380
                       ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14: 1997 tcaagttccttcctctggctggtaccagattttagtgttaattttctcccagcatgtgat 1938

AB24#9          : 381  ttctaagaacgttttaattcatgatgtcccattgtcccaaganttattaataactgaagg 440
                       |||||||||||||||||||||||||||| |||||||||||||| |   ||||||||||||||
Mouse Cyp4a14: 1937 ttctaagaacgttttaattcatgatgt-ccattgtcccaagagt--ctaataactgaagg 1881

AB24#9          : 441  gagagaaaggtacaagtgtctccccctctaaaatatatc 479
                       |||||||||||||||||||||  ||||||||||||||||
Mouse Cyp4a14: 1880 gagagaaaggtacaagtgtct--ccctctaaaatatatc 1844
```

B 26.3: Summary of sequence alignment of cDNA subclone **AB24#9** with **mouse Cyp4a14**

FluoroDD gel AB (AP3 + ARP3)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AB24	-	+	-	-	420	AB24#9	460	M13 reverse	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 14 (Cyp4a14) (2547 bp)	e-165 331/339 (97%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 27.1: DNA sequence of cDNA subclone AB25#6 (AP3 & ARP3) using M13 forward (-20) primer

```
  1 AACTCACTCA TACGGGNCAN TCNGGGCCCN CTAGNATGCN TGCTCGAGCG GCCGCCAGTG
 61 TGATGGATAT CTGCAGAATN CNGCCCTTAG CGGATAACAA TTCTCACACA GGAGACCATT   ARP3
121 GCACTGACAG AGGAAGATAT ATCCTNCAGA GGGAGACACT TGTACCTTTC TCTCCCTTCA
181 GTTATTANCA CTCTTGGGAC AATGGACATC ATGAATTAAA ACGTNCNTTA GAAATCACCA
241 TGCTGGGAGA AAATTAACCA CCTAAAATCT GGTACCCAGC CCAGCAGGNA AGGGAACTTG
301 CACCTCACAA ATAANGAGNA TTNNTTANCA TATTNCCTGT CNTGGTCTCA TAGTTAAAGT
361 TAATGTTTTC CCTGCCTTTC TGGGCATATG CCCCTCATCT TTTTCNTATN NAAGNTAANT
421 TAANTAACCT GNANTAACNA AGAAAAAGGG TTAAAAAGAA GNAAAAATTG NAANTTANGN
481 TTTTTTTGGN CCNNNCTTTT NGGGGNCCCA AAANNTGGTN NAAAAAAAAT TCCATNTTTN
541 ATTTCTAACC AAAAAAAAAA AAAAGCCCCT ATNAGTGNAG CTCNNTTATT AAAAAGGGGG
601 CGAAAATNNC CCANCCCACC NCNTGGGGNC NNGGGGCNCG TTTAAANNTT AAANTTGGGG
661 NGATTCNCCC GGAAAAGNCN TCCCNGNNGG GTTNAACCNC NAAAAAAACA ANCANAAGAA
721 AATNTNTGNN CCNNTTNANN CCACCCCTTT GNGNNAAGNG TTTNATTTTT TCCTTTCNTT
781 TTTATNTNNG TTTNGTGTNT TNTTCANANN CNNTTTNAAN AAAANNNTTA TNNNGNCNNT
841 TTTNTGGGGG NGNNNGNNGN NTTNNNNAAA TTTTTAAANT TNTGGGGGGN NTTTNCNNTT
901 TNNNTANNCC CTNNNNGGTT TTNTTNNCCN TTNGGTGTTG GTGTTNGAAN AAAATTNTTN
961 GGTTTTNNNN NN
```

B 27.2: Sequencing alignment of cDNA subclone **AB25#6** with **mouse Cyp4a14** by BLAST searching against the National Center for Biotechnology Information database

```
AB25#6          : 115  accattgcactgacagaggaagatatatcctncagagggagacacttgtacctttctctc 174
                       ||||||||||||||||||||||||||||||  |  ||||||||||||||||||||||||||
Mouse Cyp4a14: 1823 accattgcactgacagaggaagatatat--tttagagggagacacttgtacctttctctc 1880

AB25#6          : 175  ccttcagttattancactcttgggacaatggacatcatgaattaaaacgtncnttagaaa 234
                       |||||||||||||  ||||||||||||||||||||||||||||||||||| | |||||||
Mouse Cyp4a14: 1881 ccttcagttattag-actcttgggacaatggacatcatgaattaaaacgttc-ttagaaa 1938

AB25#6          : 235  tcaccatgctgggagaaaattaaccacctaaaatctggtacc 276
                       ||| ||||||||||||||||||||   ||||||||||||||||
Mouse Cyp4a14: 1939 tca-catgctgggagaaaattaac--actaaaatctggtacc 1977
```

B 27.3: Summary of sequence alignment of cDNA subclone **AB25#6** with **mouse Cyp4a14**

FluoroDD gel AB (AP3 + ARP3)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AB25	-	+	-	-	405	AB25#6	450	M13 forward (-20)	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 14 (Cyp4a14) (2547 bp)	7e-47 150/162 (92%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 28.1: DNA sequence of cDNA subclone AB26#17 (AP3 & ARP3) using M13 forward (-20) primer

```
  1 TAATACGACT CACTATAGGG CGAATTGGGC CCTCTAGATG CATGCTCGAG CGGCCGCCAG
 61 TGTGATGGAT ATCTGCAGAA TTCGGCTTAG CGGATAACAA TTTCACACAG GAGACCATTG   ARP3
121 CACTGACAGA GGAAGATATA TTTTAGAGGG AGACACTTGT ACCTTTCTCT CCCTTCAGTT
181 ATTAGACTCT TGGGACAATG GACATCATGA ATTAAAACGT TCTTAGAAAT CACATGCTGG
241 GAGAAAATTA ACACTAAAAT CTGGTACCAG CCAGAGGAAG GAACTTGACT CAAAATAAGA
301 GATTTTTAGA TATTTCTGTC TGTCTCATAG TTAAAATTAA TGTTTTCCTG CTTTCTGGCA
361 TATGCCTCAT CTTTTCTATG AAGTAGTAAT ACTGATACAG AAAGGTAGAG AGAAATGAAT
421 AGTTTTTGCT ACTTTGGGCC AAACTGTGAA AAAATNCATT TTATTTCACC AAAAAAAAAG
481 CCCTATAGTG AGTCGTATTA CAAGCNGAAT NCCAGCACAC TGGCGGCCGT AACTAGTGNA
541 TNCGAGCTCN GTANCAAGCT GGGCGTAATC ATGGTCAAGC TGTTTCTGTG TGAANTGTAT
601 CGCTCNCANT CCCACAACTA CGANCCGANC TAAGTGTAAG CNNNNNGCTA TNNACTACTC
661 ATANTGNNCC CTACGCGNTC GTNGAACGG
```

B 28.2: Sequencing alignment of cDNA subclone **AB26#17** with **mouse Cyp4a14** by BLAST searching against the National Center for Biotechnology Information database

```
AB26#17       : 114  accattgcactgacagaggaagatatattttagagggagacacttgtacctttctctccc 173
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14: 1823 accattgcactgacagaggaagatatattttagagggagacacttgtacctttctctccc 1882

AB26#17       : 174  ttcagttattagactcttgggacaatggacatcatgaattaaaacgttcttagaaatcac 233
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14: 1883 ttcagttattagactcttgggacaatggacatcatgaattaaaacgttcttagaaatcac 1942

AB26#17       : 234  atgctgggagaaaattaacactaaaatctggtaccagccagaggaaggaacttgactcaa 293
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14: 1943 atgctgggagaaaattaacactaaaatctggtaccagccagaggaaggaacttgactcaa 2002

AB26#17       : 294  aataagagatttttagatatttctgtctgtctcatagttaaaattaatgttttcctgctt 353
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14: 2003 aataagagatttttagatatttctgtctgtctcatagttaaaattaatgttttcctgctt 2062

AB26#17       : 354  tctggcatatgcctcatcttttctatgaagtagtaatactgatacagaaaggtagagaga 413
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14: 2063 tctggcatatgcctcatcttttctatgaagtagtaatactgatacagaaaggtagagaga 2122

AB26#17       : 414  aatgaatagtttttgctactttgggccaaactgtgaaaaaatncattttatttca 468
                     |||||||||||||||||||||||||||||||||||||||| ||||||||||||
Mouse Cyp4a14: 2123 aatgaatagtttttgctactttgggccaaactgtgaaaaaatccattttatttca 2177
```

B 28.3: Summary of sequence alignment of cDNA subclone **AB26#17** with **mouse Cyp4a14**

FluoroDD gel AB (AP3 + ARP3)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AB26	-	+	-	-	390	AB26#17	450	M13 forward (-20)	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 14 (Cyp4a14) (2547 bp)	0 354/355 (99%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 29.1: DNA sequence of cDNA subclone AB26#30 (AP3 & ARP3) using M13 reverse primer

```
  1 TACCAAGCTT GAGTACCGAG CTCNGATCCA CTAGTAACGG CCGCCAGTGT GCTGGAATTC
 61 GGCTTGATAA TACGACCCAC TATAGGGCTT TTTTTTTTG GTGAAATAAA ATGGATTTTT   AP3*
121 TCACAGTTTG GCCCAAAGTA GCAAAAACTA TTCATTTCTC TCTACCTTTC TGTATCAGTA
181 TTACTACTTC ATAGAAAAGA TGAGGCATAT GCCAAAAAGC AGGAAAACAT TAATTTTAAC
241 TATGAGACAG ACAGAAATAT CTAAAAATCT CTTATTTTGA GTCAAGTTCC TTCCTCTGGC
301 TGGTACCAGA TTTTAGTGTT AATTTTCTCC CAACATGTGA TTTCTAAGAA CGTTTTAATT
361 CATGATGTCC ATTGTCCCAA AGAGTCTAAT AACTGAAGGG AGAGAAAGGT ACAAGTGTCT
421 CCCTCTAAAA TATATCTTCC TCTGTCAGTG CAATGGTCTC CTGTGTGAAA TTGTNATCCG
481 CTAAGCCGAA TTCTGCAGAT ATNCATCACA CTGGCGGCCG CTCGAGCATG CATCTAGAGG
541 CCCAATNCGC NNTATAGTGA GTCGTATTAC AATTCACTGC CNGTCGTNTN ACAACGTCGT
601 GACTGGNANA ACCCNGGCGT NANCCACCNT ANTCGCNTGC AGCNCANTCC CCTTTCGCNA
661 CTGNNGTNNA CGAGANGCNN NNNGATNCCC TCCACAGTGC CNNCGTG
```

B 29.2: Sequencing alignment of cDNA subclone **AB26#30** with **mouse Cyp4a14** by BLAST searching against the National Center for Biotechnology Information database

```
AB26#30       : 102  tgaaataaaatggatttttcacagtttggcccaaagtagcaaaaactattcatttctct 161
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14: 2177 tgaaataaaatggatttttcacagtttggcccaaagtagcaaaaactattcatttctct 2118

AB26#30       : 162  ctacctttctgtatcagtattactacttcatagaaaagatgaggcatatgccaaaaagca 221
                     |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Mouse Cyp4a14: 2117 ctacctttctgtatcagtattactacttcatagaaaagatgaggcatatgccagaaagca 2058

AB26#30       : 222  ggaaaacattaattttaactatgagacagacagaaatatctaaaaatctcttattttgag 281
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14: 2057 ggaaaacattaattttaactatgagacagacagaaatatctaaaaatctcttattttgag 1998

AB26#30       : 282  tcaagttccttcctctggctggtaccagattttagtgttaattttctcccaacatgtgat 341
                     |||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||
Mouse Cyp4a14: 1997 tcaagttccttcctctggctggtaccagattttagtgttaattttctcccagcatgtgat 1938

AB26#30       : 342  ttctaagaacgttttaattcatgatgtccattgtcccaaagagtctaataactgaaggga 401
                     |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Mouse Cyp4a14: 1937 ttctaagaacgttttaattcatgatgtccattgtccc-aagagtctaataactgaaggga 1879

AB26#30       : 402  gagaaaggtacaagtgtctccctctaaaatatatcttcctctgtcagtgcaatggt 457
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a14: 1878 gagaaaggtacaagtgtctccctctaaaatatatcttcctctgtcagtgcaatggt 1823
```

B 29.3: Summary of sequence alignment of cDNA subclone **AB26#30** with **mouse Cyp4a14**

FluoroDD gel AB (AP3 + ARP3)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AB26	-	+	-	-	390	AB26#30	450	M13 reverse	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 14 (Cyp4a14) (2547 bp)	0 353/356 (99%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 30.1: DNA sequence of cDNA subclone AB29#7 (AP3 & ARP3) using M13 forward (-20) primer

```
  1 NCNNTCACNT ATAGGGCGAA TTGGGCCCTC TAGATGCNTG CTCGAGCGGC CGCCAGTGTG
 61 ATGGATATCT GCAGAATTCG CCCTTGTAAT ACGACTCACT ATAGGGCTTT TTTTTTTGG    AP3*
121 GACAGGGTTT CTCGGTGCAG CCCTGGCTGT CCTGGAACTC ACTGTGTAGA CCAGGCTGGC
181 CTCGAACTCA GAGATCACCT GCCTCTGCCT CTGGACTGCT AAGATTAAAG GTGTACAACA
241 CCACCACCTG ATTAAATAGT TTAAACAAAC AAACAACAAC AACAAAAACC AGAAGAAAAG
301 AGAGCTGGAG AGATGGCTCA GCAGTTAAGA GCACCAACTG CTCTTCCAGA GGTCCTGAGT
361 TCAATTCCCA GCAACCACAT GGTGGCTCCC AACCATCTGC AATGGTCTCC TGTGTGAAAT
421 GGTTATCCGC TAAGGGCGAA TTCCAGCACA CTGGCGGCCG TTACTAGTGG ATCCGAGCTC
481 GGTACCAAGC TTGATGCATA GCTTGAGTAT NCTATAGTGT CACCTAAATA GCTTGGCGTA
541 ATCATGGTCA TAGCTGTTTC CTGTGTGAAA TTGTNATCCG CTCACAATTN CACACAACAT
601 ACGAGCCGGA AGCATAAAGT GTAAAGCCTG GGGTGCCTAA TGAGTGAGCT AACTCACATN
661 AATNGCGTNG CGCTCACTGC CCGCTTTCCA GTCNGGAAAC CTGTCGTGCA GCTGCATTAA
721 TGAATNNCCA CGCGCGGGAN NNCGGNNGCG TNTNGGCNCT CTCCNCTCCC GCTCCTGACT
781 GCTGCCTNNT GTTGGNNNNC NNN
```

B 30.2: Sequencing alignment of cDNA subclone **AB29#7** with **mouse catalase** by BLAST searching against the National Center for Biotechnology Information database

```
AB29#7          : 439 aattccagcacactggcggccgttactagtggatccgagctcggtaccaagct-tgatgc 497
                      ||||||||||||||||||||||||||||||||||||||||||||||||||| | ||||||
Mouse catalase: 31  aattccagcacactggcggccgttactagtggatccgagctcggtaccaaggtgtgatgc 90

AB29#7          : 498 atagcttgagtatnctatagtgtcacctaaatagcttggcgtaatcatggtcatagctgt 557
                      |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Mouse catalase: 91  atagcttgagtattctatagtgtcacctaaatagcttggcgtaatcatggtcatagctgt 150

AB29#7          : 558 ttcctgtgtgaaattgtnatccgctcacaattncacacaacatacgagccggaagcataa 617
                      |||||||||||||||| |||||||||||||| ||||||||||||||||||||||||||||
Mouse catalase: 151 ttcctgtgtgaaattgttatccgctcacaattccacacaacatacgagccggaagcataa 210

AB29#7          : 618 agtgtaaagcctggggtgcctaatgagtgagctaactcacatnaatngcgtngcgctcac 677
                      |||||||||||||||||||||||||||||||||  || ||| ||| |||| ||||||||
Mouse catalase: 211 agtgtaaagcctggggtgcctaatgagtgagctatttctcattaattgcgttgcgctcac 270

AB29#7          : 678 tgcccgctttcca 690
                      |||||||||||||
Mouse catalase: 271 tgcccgctttcca 283
```

B 30.3: Summary of sequence alignment of cDNA subclone **AB29#7** with **mouse catalase**

FluoroDD gel AB (AP3 + ARP3)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AB29	-	+	-	-	335	AB29#7	380	M13 forward (-20)	if84a04.x1 Kaestner ngn3 - - subtracted *Mus musculus* cDNA 3' similar to SW:CATA_CAMJE Q59296 CATALASE (283 bp)	e-114 242/253 (95%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 31.1: DNA sequence of cDNA subclone AC1#1 (AP2 & ARP19) using M13 forward (-20) primer

```
  1 ACCCTNACTA TAGGGCGAAT TGGGCCCTCT AGATGCATGC TCGAGCGGCC GCCAGTGTGA
 61 TGGATATCTG CAGAATTCGC CCTTGTAATA CGACTCACTA TAGGGCTTTT TTTTTTGCCC   AP2*
121 GGCCCATTCA TTCTTTATTC AGGTGGCATA AAAATCACTA CAAAAACTTT ACAAAAGAGT
181 CTTGGGAGCT AAAGGGTCCC TTCCTTGCCT CAGTCCCCAA GATTCCTGGC AGGGGAGGAC
241 AAGAGAGAGA AGAAGGAGGA AGACTCCTGG CAGTGTTGGC ATCTCCAAAT ACCAGAGGGG
301 TGACTTGGGT GACAGGACAC AGGTNGGGGA CCTGAATGTC TTCAGCAAGG GACACTCTTG
361 TAGGGTAGGT CAGCCTCCAA CCATGAAGTA TAACACCCAA GGCCAGTCTA AGCTTGGGAG
421 ACCAACACTT GTCTCTCCTT TTCCCCACCC AGGGTGTCTG GAATATGTCT AAAGATGGCC
481 TCTCCAGCCT CTGCTTACAA ATGTGGAGGN NCCCTAAGTT AGGGACTTGC CTAACCTACC
541 TCTAGCCCAA ACTGTGTNCA CAAGTGCCAG CCCACAAAAG ATCACCCCCT GAGCCCCTTG
601 GGNAAGAAAT GAAGATTCCC CATGNCCTGC CNNNCCTCCA GGCCCCCACC CCCNCCTGGC
661 TGCNNANAAA ACNNGNTTNT ANNACTGNTG NATGGTTCNT TCCNGNTCCC ACCCTTATCC
721 CCCCAAAGCN GGNTAAAAAA GAGTTNNAGG AGCNGAAAGN TGGTCAGGTN GGACTAAGAT
781 TTNNTGNNGG NCNNTNNNNT GGGGGANNNG AATGGGNNNN
```

B 31.2: Sequencing alignment of cDNA subclone **AC1#1** with **mouse serine (or cysteine) proteinase inhibitor (SPI)** by BLAST searching against the National Center for Biotechnology Information database

```
AC1#1     : 122  gcccattcattctttattcaggtggcataaaaatcactacaaaaactttacaaaagagtc 181
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 2166 gcccattcattctttattcaggtggcataaaaatcactacaaaaactttacaaaagagtc 2107

AC1#1     : 182  ttgggagctaaagggtcccttccttgcctcagtccccaagattcctggcaggggaggaca 241
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 2106 ttgggagctaaagggtcccttccttgcctcagtccccaagattcctggcaggggaggaca 2047

AC1#1     : 242  agagagagaagaaggaggaagactcctggcagtgttggcatctccaaataccagaggggt 301
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 2046 agagagagaagaaggaggaagactcctggcagtgttggcatctccaaataccagaggggt 1987

AC1#1     : 302  gacttgggtgacaggacacaggtnggggacctgaatgtcttcagcaagggacactcttgt 361
                 |||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Mouse SPI: 1986 gacttgggtgacaggacacaggttggggacctgaatgtcttcagcaagggacactcttgt 1927

AC1#1     : 362  agggtaggtcagcctccaaccatgaagtataacacccaaggccagtctaagcttgggaga 421
                 |||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Mouse SPI: 1926 agggtaggtcagcctccaaccatgaagtataaca-ccaaggccagtctaagcttgggaga 1868

AC1#1     : 422  ccaacacttgtctctcctttccccacccagggtgtctggaatatgtctaaagatggcct 481
                 |||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Mouse SPI: 1867 ccaacacttgtctctcctttt-cccacccagggtgtctggaatatgtctaaagatggcct 1809

AC1#1     : 482  ctccagcctctgcttacaaatgtggaggnnccctaagttagggacttgcctaacctacct 541
                 |||||||||||||||||||||||||||||||| |||||||||||||||||||||||||||||
Mouse SPI: 1808 ctccagcctctgcttacaaatgtggagggaccctaagttagggacttgcctaacctacct 1749

AC1#1     : 542  ctagcccaaactgtgtncacaagtgccagcccacaaaagatcacccccctgagcccccttgg 601
                 |||||| |||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1748 ctagccaaaactgtgtccacaagtgccagcccacaaaagatcacccccctgagcccccttgg 1689

AC1#1     : 602  gnaagaaatgaagattccccatg 624
                 | ||||||||||||||||||||||
Mouse SPI: 1688 g-aagaaatgaagattccccatg 1667
```

B 31.3: Summary of sequence alignment of cDNA subclone **AC1#1** with **mouse SPI**

FluoroDD gel AC (AP2 + ARP19)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AC1	+	-	+	+	1800	AC1#1	1830	M13 forward (-20)	*Mus musculus* serine (or cysteine) proteinase inhibitor, clade F, member 2 (Serpinf2) (2187 bp)	0 495/503 (98%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 32.1: DNA sequence of cDNA subclone AC1#1 (AP2 & ARP19) using M13 reverse primer

```
  1 CNTATTTAGG TGACACTATA GAATACTCAA GCTATGCATC CAAGCTTGGT ACCGAGCTCG
 61 GATCCACTAG ATAACGGCCG CCAGTGTGCT GGAATTCCGC CCTTAGCGGA TAACAATTTC    ARP19
121 ACACAGGATT TTGGCTCCCG CATCTACTGA GCCATTTCTA CCAGAACCTA GGCCCAGGGA
181 CAATCCGGCT GGCTGCCAGA ATATACCTGC AGAAAGGATT TCCCATCAAA GACGATTTCC
241 TGGCANGCCA CTCGGAACNG GCTCTTTGGT GCGAAGCCCN GTGNNNNACC TGACTGGACA
301 AGCAGGACGG AAGACCTGGC GACNCNNTCN CNCAATGGGT GAAGGANGGC NNAGAGGGGA
361 CAGATTGANG GATTTCTNTC TGAGCCTGCC GGATNGCCCG TGCTGTTTNT CTCACGCATC
421 CTTNANGTTT NTGGAGGANN NGTTNGACCC NNNNTNNNNN ATNNNNNTGG CTGANNNGTN
481 TGTNGTGGNT GNTNNNNGTG GTNTTNTTNT GGGTCTGNTG NNNNNNNTNG TGNNNNNNNN
541 NNNNNTNNNN TGGTNNTNNN NTNNNTNNNT TNGTNNNNNN NTNNNNNNNN NNNGGTNNNN
601 NNN
```

B 32.2: Sequencing alignment of cDNA subclone **AC1#1** with **mouse serine (or cysteine) proteinase inhibitor (SPI)** by BLAST searching against the National Center for Biotechnology Information database

```
AC1#1     : 121 acacaggattttggctcccgcatctactgagccatttctaccagaacctaggcccaggga 180
Mouse SPI: 439 acacaggatcctgcctcccgcatctactgagccatttctaccagaacctaggcccaggga 498

AC1#1     : 181 caatccggctggctgccagaatatacctgcagaaaggatttcccatcaaagacgatttcc 240
Mouse SPI: 499 caatccgactggctgccagaatatacctgcagaaaggatttcccatcaaagacgatttcc 558

AC1#1     : 241 tgg 243
Mouse SPI: 559 tgg 561
```

B 32.3: Summary of sequence alignment of cDNA subclone **AC1#1** with **mouse SPI**

FluoroDD gel AC (AP2 + ARP19)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AC1	+	-	+	+	1800	AC1#1	1830	M13 reverse	*Mus musculus* serine (or cysteine) proteinase inhibitor, clade F, member 2 (Serpinf2) (2187 bp)	7e-52 119/123 (96%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 33.1: DNA sequence of cDNA subclone AC1#2 (AP2& ARP19) using M13 forward (-20) primer

```
  1 GGGCCCTCTA GATGCATGCT CGAGCGGCCG CCAGTGTGAT GGATATCTGC AGAATTCGCC
 61 CTTGTAATAC GACTCACTAT AGGGCTTTTT TTTTGCTGTA GCATCAGGCA CGAGCCAGGC   AP2*
121 CAGCCTGTGT TTAATGGAAG GAACCCAATT CAGAAGGAAG GATGACATTT TAGGGTGGTC
181 ATTTATGTGT GGGATCTACC ACTTTTCCCA CAAAGATGGG GCTCTGAGTG TGTTCTTCAA
241 ATATTATGAA AAGGAAAGGG TGGTCNAAGC GCAGGATAGG GGGCATAGAC ATAGGAACCA
301 TTTGTAAGAC TGTAACTGCT GCAGCTTCTG TTCCTGTCTC ATCGATGGTC AGCACAGCCT
361 TATGCACAGC CCTGGCTGAG CTTTCCAGGG GAGCATTCNT CCCTCTGTGA TTCCGGAGAG
421 GTCAGCCCCC ATTGTTGAAG ATCCGGGTGA TGCCCCAGTN GGACTNATGA GTGTTTTNAA
481 GNNATATTCT CCAGAGATGG GACAGTCTGG GGAAGTTNGA TNNTGGGGGC TNCCCCTTTN
541 TGNGNCNTGN TNNNAGCNGG GAACNTTAAA ANNATGAAGC CTCNTTNGNN TGAAAAGTTT
601 TGGCTCCAAN ATGCTGCCTT TTTCCNNTNT TNNTTGGGGC CAAAANNGAA NNNCAGCACT
661 TGGGTTTNNN NTTGCNNTNN TTCCTTTTCC CCGCCAACCC ACCCTGNAAC GGCTTTCGTG
721 ACCNTGGNTT CNNCNTTCNC CCCTTNCACC GAAAANGGTN NNNNNNNNNN NNNNNNNNNN
781 NNNN
```

B 33.2: Sequencing alignment of cDNA subclone **AC1#2** with **mouse serine (or cysteine) proteinase inhibitor (SPI)** by BLAST searching against the National Center for Biotechnology Information database

```
AC1#2     : 95   gctgtagcatcaggcacgagccaggccagcctgtgtttaatggaaggaacccaattcaga 154
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1376 gctgtagcatcaggcacgagccaggccagcctgtgtttaatggaaggaacccaattcaga 1317

AC1#2     : 155  aggaaggatgacattttagggtggtcatttatgtgtgggatctaccacttttcccacaaa 214
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1316 aggaaggatgacattttagggtggtcatttatgtgtgggatctaccacttttcccacaaa 1257

AC1#2     : 215  gatggggctctgagtgtgttcttcaaatattatgaaaaggaaaggggtggtcnaagcgcag 274
                 ||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||
Mouse SPI: 1256 gatggggctctgagtgtgttcttcaaatattatgaaaaggaaaggggtggtcgaagcgcag 1197

AC1#2     : 275  gataggggggcatagacataggaaccatttgtaagactgtaactgctgcagcttctgttcc 334
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1196 gataggggggcatagacataggaaccatttgtaagactgtaactgctgcagcttctgttcc 1137

AC1#2     : 335  tgtctcatcgatggtcagcacagccttatgcacagccctggctgagctttccaggggagc 394
                 |||||||||||||||||||||||||||||||||| |||||||||||| ||||||||||
Mouse SPI: 1136 tgtctcatcgatggtcagcacagccttatgcacag-cctggctgagctt--caggggagc 1080

AC1#2     : 395  attcntccctctgtgattccggagaggtcagccccattgttgaagatccgggtgatgcc 454
                 ||||  |||||||||||||||||||||| |||||||||||||||||||||||||| |
Mouse SPI: 1079 attct--cctctgtgattccggagaggtcag-ccccattgttgaagatccgggtgatg-c 1024

AC1#2     : 455  ccagtnggactnatgagtgttttnaagnnatattctccagagatgggacagtctggggaa 514
                 ||||| ||||| |||||||| || ||| ||||||||||||||| ||||||||||||||||
Mouse SPI: 1023 ccagt-ggactcatgagtgtcttcaagttatattctccagagat-ggacagtctggggaa 966

AC1#2     : 515  gt 516
                 ||
Mouse SPI: 965  gt 964
```

B 33.3: Summary of sequence alignment of cDNA subclone **AC1#2** with **mouse SPI**

FluoroDD gel AC (AP2 + ARP19)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AC1	+	-	+	+	1800	AC1#2	1600	M13 forward (-20)	*Mus musculus* serine (or cysteine) proteinase inhibitor clade A, member 1a (SPI) (1393 bp)	0 406/422 (96%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 34.1: DNA sequence of cDNA subclone AC1#2 (AP2& ARP19) using M13 reverse primer

```
  1 GATACTCAAG CTATGCNTCA AGCTTGGTAC CGAGACTCGG ATCCACTAGT AACGGCCGCC
 61 AGTGTGCTGG AATTCGCCCT TAGACGGATA ACAATTTCAC ACAGGATTTT GGCTCCTACT   ARP19
121 GCTTAAATAC AGACTAGGAC AGGGCTCTGT CTCCTCAGCC TCGGTCACCA CCCAGCTCTG
181 GGACAGCAAG CTGAAAATGA CTCCCTCCAT CTCATGGGGT CTACTGCTTC TGGCAGGCCT
241 GTGTTGCCTG GTCCCCAGCT TTCTGGCTGA GGATGTTCAG GAGACAGACA CCTCCCAGAA
301 GGATCAGTCC CCCCAGCCTC CCATGAGATC GCCTACAAAC CCTGGGAGAC TTTGCAATCA
361 GCCTATACCG GGAGCTGGTC CATCAGTNCN AACACTTTCC AACCATNNTT CTTCTCCCNN
421 AGTGAGCCAT NNGCNACACC CNTTTGCNTA TGCTCTCCCT ANGGGANNCN AGGGTGACNN
481 TNCNNNCCGA TCTAAGGGCT GANTNNNNNN NNTNNNGTNN NNTNTNNNCT NNNNNNANNN
541 NCNNNNNNNT GGNTNNTNNN NNCNNTNNNN NNNNNNNNNN NN
```

B 34.2: Sequencing alignment of cDNA subclone **AC1#2** with **mouse serine (or cysteine) proteinase inhibitor (SPI)** by BLAST searching against the National Center for Biotechnology Information database

```
AC1#2     : 147 ctgtctcctcagcctcggtcaccacccagctctgggacagcaagctgaaaatgactccct 206
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1   ctgtctcctcagcctcggtcaccacccagctctgggacagcaagctgaaaatgactccct 60

AC1#2     : 207 ccatctcatggggtctactgcttctggcaggcctgtgttgcctggtccccagctttctgg 266
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 61  ccatctcatggggtctactgcttctggcaggcctgtgttgcctggtccccagctttctgg 120

AC1#2     : 267 ctgaggatgttcaggagacagacacctcccagaaggatcagtccccccagcctcccatga 326
                ||||||||||||||||||||||||||||||||||||||||  ||||||||||||||||
Mouse SPI: 121 ctgaggatgttcaggagacagacacctcccagaaggatcagt--ccccagcctcccatga 178

AC1#2     : 327 gatcgcctacaaaccctgggagactttgcaatcagcctataccgggagctggtccatcag 386
                |||||  |||||||  ||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 179 gatcg-ctacaaa-cctgggagactttgcaatcagcctataccgggagctggtccatcag 236

AC1#2     : 387 t 387
                |
Mouse SPI: 237 t 237
```

B 34.3: Summary of sequence alignment of cDNA subclone **AC1#2** with **mouse SPI**

FluoroDD gel AC (AP2 + ARP19)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AC1	+	-	+	+	1800	AC1#2	1600	M13 reverse	*Mus musculus* serine (or cysteine) proteinase inhibitor clade A, member 1a (SPI) (1393 bp)	e-116 237/241 (98%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 35.1: DNA sequence of cDNA subclone AC2#2 (AP2 & ARP19) using M13 reverse primer

```
  1 ANGCCTATTT AGGTGACNCT ATAGAATACT CNAGACTATG CATCAAGCTT GGTACCGAGC
 61 TCGGATCCAC TAGTAACGGC CGCCAGTGTG CTGGAATTCG CCCTTAGCGG ATAACAATTT   ARP19
121 CACACAGGAT TTTGGCTCCC CTATTACAAC CAGGGCTATT TCTTGATAGA GGAAGGTAGT
181 AAGCCAGAGG ATGTAGATGG GGTCTTAGAA GAGTNTGGTT TTAGAATGGG ACCCTTCAGG
241 GTGTCTGACC TCGCAGGGCT AGATGTGGGT TGGAAAGTNC GCAAAGGGCA AGGCCTTACT
301 GGACCGTCCT TACCTCCAGG AACCCCCACC CGAAAGAGGG GCAATACCAG GTATCCCCCA
361 ATTGCTGATA TGCTCTGTGA AGCTGGGCGA TTTGGTCAGA AGACAGGTAA GGGCTGGTAT
421 CAGTATGACA AGCCACTGGG TCGCATCCAC AAACCTGATC CCTGGCTTTC TGAGTTTCTG
481 TCACAGTATA GAGAAACCCA TCACATCAAG CAGCGCTCCA TCAGCAAGGA GGAAATCCTG
541 GAGCGTNGCT TATATTCCCT TATCAACGAG GCATCCCGCA TNTNGGANGA GGGGATGGCC
601 GCTAGCCCAG AGCACANNAT GTCATCTACT GCATGGGTAT NGNTGGCCAN GCACGTGGTG
661 GGCCATGTAC TATGCTGCNC AGTGGGCTGC CACAGNNTAA GANNTGCAGA ATATANCACC
721 NACTCCGACN NCCCACNCTG ACCCNNGACN CNNGANGAGG GGNTNNCNNN GANNCCCCCC
781 TGANNANGGA AAGNTGNNGA ANNATAT
```

B 35.2: Sequencing alignment of cDNA subclone **AC2#2** with **mouse bifunctional enzyme (PBFE)** by BLAST searching against the National Center for Biotechnology Information database

```
AC2#2      : 139  ccctattacaaccagggctatttcttgatagaggaaggtagtaagccagaggatgtagat 198
                  ||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Mouse PBFE: 1469 ccctattacaaccagggctatttcttgatagaggaaggtagtaagccagagggtgtagat 1528

AC2#2      : 199  ggggtcttagaagagtntggttttagaatgggacccttcagggtgtctgacctcgcaggg 258
                  |||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Mouse PBFE: 1529 ggggtcttagaagagtttggttttagaatgggacccttcagggtgtctgacctcgcaggg 1588

AC2#2      : 259  ctagatgtgggttggaaagtncgcaaagggcaaggccttactggaccgtccttacctcca 318
                  |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Mouse PBFE: 1589 ctagatgtgggttggaaagttcgcaaagggcaaggccttactggaccgtccttacctcca 1648

AC2#2      : 319  ggaacccccacccgaaagaggggcaataccaggtatccccaattgctgatatgctctgt 378
                  |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Mouse PBFE: 1649 ggaacccccacccgaaagaggggcaataccaggtactccccaattgctgatatgctctgt 1708

AC2#2      : 379  gaagctgggcgatttggtcagaagacaggtaagggctggtatcagtatgacaagccactg 438
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse PBFE: 1709 gaagctgggcgatttggtcagaagacaggtaagggctggtatcagtatgacaagccactg 1768

AC2#2      : 439  ggtcgcatccacaaacctgatccctggctttctgagtttctgtcacagtatagagaaacc 498
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse PBFE: 1769 ggtcgcatccacaaacctgatccctggctttctgagtttctgtcacagtatagagaaacc 1828

AC2#2      : 499  catcacatcaagcagcgctccatcagcaaggaggaaatcctggagcgtngcttatattcc 558
                  |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Mouse PBFE: 1829 catcacatcaagcagcgctccatcagcaaggaggaaatcctggagcgttgcttatattcc 1888

AC2#2      : 559  cttatcaacgaggcatcccgcatntnggangaggggatggccgctagcccagagcaca-n 617
                  |||||||||||||||| |||||| | ||| ||||||||||||||||||||||||||| 
Mouse PBFE: 1889 cttatcaacgaggcattccgcatcttggaggaggggatggccgctagcccagagcacatt 1948

AC2#2      : 618  natgtcatctac-tgcatgggtatngntggcc-angcacgt-ggtggg-ccatgtactat 673
                   |||||||||||| |||||||||| | ||||| | |||||| |||||| ||||||||||| 
Mouse PBFE: 1949 gatgtcatctacttgcatgggtatgggtggccaaggcacgtgggtgggcccatgtactat 2008

AC2#2      : 674  gctgc 678
                  |||||
Mouse PBFE: 2009 gctgc 2013
```

B 35.3: Summary of sequence alignment of cDNA subclone **AC2#2** with **mouse PBFE**

FluoroDD gel AC (AP2 + ARP19)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AC2	-	+	-	-	1600	AC2#2	1630	M13 reverse	*Mus musculus* peroxisomal bifunctional enzyme (PBE) (PBFE) [includes: enoyl-coA hydratase (EC 4.2.1.17); 3,2-trans_enoyl-coA isomerase(EC 5.3.3.8); 3-hydroxyacyl-coA dehydrogenase (EC 1.1.1.35)] (2979 bp)	0 525/545 (96%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 36.1: DNA sequence of cDNA subclone AC2#5 (AP2 & ARP19) using M13 reverse primer

```
  1 ACTATTTAGG TGACACTATA GAATACTACA AGACTATGAC ATACAAGACT TGGTACCGAG
 61 ACTCGGATCC ACTAGTAACG GCCGCCAGTG TGCTGGAATT NCGCCCNTTA GACGGATAAC   ARP19
121 AATTTCACAC AGGATTTTGG CTCCAGGGAC ACTNNATGCA TGATATGGCA AATANNNNAG
181 CAGGGAGGCA CTCCTGTGGG CTTAAGCACC TAAACCTTTT CAAATTCTCT ATGGACACAC
241 TGATGAGATA CTGAAGCGTG GGTATCAGCA CTGAACTCCA CNNGNNANNA TCAGGGGTCN
301 AGGNNTGGCA CTGTGATNNT CCATACCGTT CAGAAAGGTC NGTACNTGAG ANCCCTACGA
361 CTGCCTTGNG AGAGTTCNNT GTTCCTGACA ATTCCCAGGT TTGGGCCCTN TTCCGGGGAA
421 AANACCCCNT TGGTTGTTNT AATTCCCGNC NTGGGNNAGN AGGAANNGTC CGNTTCCCNC
481 CAAAGGGAAT NNAAGAAANN CNNNAATTTA NCACATTTTG GNNTTTTTTT NNTTANTTAA
541 ANATGNGGNN NAAAGGTTAA TNNNTGGGGG GGGGGGTNTT TCCNANNGTT TTCCCCTGAA
601 AGCTGTGNNA NCACAAANAT TNNTTNNANN NCCNNTGGTT GGCCNNTNNA NNTNGGGAAA
661 NAAACCACAA NNTTAATNNA AANNNNNNNN NNNAGNNCTT AAAANNAAAG GGGGGGNNCC
721 NNNTNNCNNG NNTTTTTTAA NNTNNNNNAN NNAAAGACCN TNNCACCANN GGGCNTNNNA
781 ANAAGCNNNN NNNNTTTTNN TNNNNTTTTN NNNNNNNNNN NNNANANNNN NNNNNNTATA
841 TTNTTANNNN NNNNGNNNN
```

B 36.2: Sequencing alignment of cDNA subclone **AC2#5** with **mouse catalase** by BLAST searching against the National Center for Biotechnology Information database

```
AC2#5          : 2   ctatttaggtgacactatagaatactacaagactatgacatacaagacttggtaccgaga 61
                     ||||||||||||||||||||||||| |||| ||||| ||| |||| ||||||||||||
Mouse catalse: 123 ctatttaggtgacactatagaatact-caag-ctatg-cat-caag-cttggtaccgag- 70

AC2#5          : 62  ctcggatccactagtaacggccgccagtgtgctggaatt 100
                     ||||||||||||||||||||||||||||||||||||||||
Mouse catalse: 69  ctcggatccactagtaacggccgccagtgtgctggaatt 31
```

B 36.3: Summary of sequence alignment of cDNA subclone **AC2#5** with **mouse catalase**

FluoroDD gel AC (AP2 + ARP19)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AC2	-	+	-	-	1600	AC2#5	1620	M13 reverse	if84h05.x1 Kaestner ngn3 - - subtracted *Mus musculus* cDNA 3' similar to SW:CATA_CAMJE Q59296 CATALASE (280 bp)	4e-19 93/99 (93%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 37.1: DNA sequence of cDNA subclone AC2#6 (AP2 & ARP19) using M13 forward (-20) primer

```
  1 NCNTCACTNN AGGGCGAATT GGGCCCTCTA GATGCATGCT CGAGCGGCCG CCAGTGTGAT
 61 GGATATCTGC AGAATTCGCC CTTGTAATAC GACTCACTAT AGGGCTTTTT TTTTTGCCCA   AP2*
121 TTCATTCTTT ATTCAGGTGG CATAAAAATC ACTACAAAAA CTTTACAAAA GAGTCTTGGG
181 AGCTAAAGGG TCCCTTCCTT GCCTCAGTCC CCAAGATTCC CGGCAGGGGA GGACAAGAGA
241 GAGAAGAAGG AGGAAGACTC CTGGCAGTGT TGGCATCTCC AAATACCAGA GGGGTGACTT
301 GGGTGACAGG ACACAGGTTG GGGACCTGAA TGTCTTCAGC AAGGGACACT CTTGTAGGGT
361 AGGTCAGCCT CCAACCATGA AGTATAACAC CAAGGCCAGT CTAAGCTTGG GAGACCAACA
421 CTTGTCTCTC CTTTTCCCAC CCAGGGTGTC TGGAATATGT CTAAAGATGG CCTCTCCAGC
481 CTCTGCTTAC AAATGTGGAG GGACCCTAAG TTAGGGACTT GCCTAACCTA CCTCTAGCCA
541 AAACTGTGTC CACAAGTGCC AGCCCACAAA AGATCACCCC CTGAGCCCCT TGGGAAGAAA
601 TGAAGATTCC CCATGCCTGC CTTCCTCCAG GCCCCACCCC ACCTGCTGCA AGAGANCAGC
661 TTCTACACTG GTGATGGTCN NTCCNGTCCC ACCCTATCCC ACAAAGCTGG TTAGANAGAG
721 TNACAGGAGC TGAGAGGCTG ATNCAGGTGG GGACTCNNAA G
```

B 37.2: Sequencing alignment of cDNA subclone **AC2#6** with **mouse serine (or cysteine) proteinase inhibitor (SPI)** by BLAST searching against the National Center for Biotechnology Information database

```
AC2#6      : 116  gcccattcattctttattcaggtggcataaaaatcactacaaaaactttacaaaagagtc 175
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 2166 gcccattcattctttattcaggtggcataaaaatcactacaaaaactttacaaaagagtc 2107

AC2#6      : 176  ttgggagctaaagggtcccttccttgcctcagtccccaagattcccggcaggggaggaca 235
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 2106 ttgggagctaaagggtcccttccttgcctcagtccccaagattcctggcaggggaggaca 2047

AC2#6      : 236  agagagagaagaaggaggaagactcctggcagtgttggcatctccaaataccagaggggt 295
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 2046 agagagagaagaaggaggaagactcctggcagtgttggcatctccaaataccagaggggt 1987

AC2#6      : 296  gacttgggtgacaggacacaggttggggacctgaatgtcttcagcaagggacactcttgt 355
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1986 gacttgggtgacaggacacaggttggggacctgaatgtcttcagcaagggacactcttgt 1927

AC2#6      : 356  agggtaggtcagcctccaaccatgaagtataacaccaaggccagtctaagcttgggagac 415
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1926 agggtaggtcagcctccaaccatgaagtataacaccaaggccagtctaagcttgggagac 1867

AC2#6      : 416  caacacttgtctctccttttcccacccagggtgtctggaatatgtctaaagatggcctct 475
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1866 caacacttgtctctccttttcccacccagggtgtctggaatatgtctaaagatggcctct 1807

AC2#6      : 476  ccagcctctgcttacaaatgtggagggaccctaagttagggacttgcctaacctacctct 535
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1806 ccagcctctgcttacaaatgtggagggaccctaagttagggacttgcctaacctacctct 1747

AC2#6      : 536  agccaaaactgtgtccacaagtgccagcccacaaaagatcacccccctgagcccctttggga 595
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1746 agccaaaactgtgtccacaagtgccagcccacaaaagatcacccccctgagcccctttggga 1687

AC2#6      : 596  agaaatgaagattccccatgcctgccttcctccaggccccaccccacctgctgcaagaga 655
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1686 agaaatgaagattccccatgcctgccttcctccaggccccaccccacctgctgcaagaga 1627

AC2#6      : 656  ncagcttctacactggtgatggtcnntccngtcccaccctatcccacaaagctggttaga 715
                   |||||||||||||||||||||  ||| ||||||||||||||||||||||||||||||
Mouse SPI: 1626 acagcttctacactggtgatggtccttccggtcccaccctatcccacaaagctggttaga 1567

AC2#6      : 716  nagagtnacaggagctgagaggctgatncaggtggggactc 756
                   ||||| |||||||||||||||||| ||||||||||||
Mouse SPI: 1566 aagagtcacaggagctgagaggctgatccaggtggggactc 1526
```

B 37.3: Summary of sequence alignment of cDNA subclone **AC2#6** with **mouse SPI**

FluoroDD gel AC (AP2 + ARP19)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AC2	-	+	-	-	1600	AC2#6	1800	M13 forward (-20)	*Mus musculus* serine (or cysteine) proteinase inhibitor, clade F, member 2 (Serpinf2) (2187 bp)	0 633/641 (98%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 38.1: DNA sequence of cDNA subclone AC4#3 (AP2 & ARP19) using M13 forward (-20) primer

```
  1 CNACNTCACT ATAGGGCGAA TTGGGCCCTC TAGATGCATG CTCGAGCGGC CGCCAGTGTG
 61 ATGGATATCT GCAGAATTCG CCCTTGTAAT ACGACTCACT ATAGGGCTTT TTTTTGCCAT   AP2*
121 AAATAATATC TACTTTTATT ATTTTATTAT CCCTATAGAC AACATAGTAT AAATTTCCCT
181 CTTCTTTGGC TACCTTTGAA ATATCCTCTG CAAGTCTACT CTGTGTNCCT CTNNTTCTTC
241 TGTACCTTTG ACCTTTTCTA AGTTTCAACC CCTGNACTTG GTCTGNNCTC AATCATTCCC
301 TTTAACCTCA TGCAGCCCAG GNTCAACGGG ACNAGAAACT CNTAGTGTAG GTTGGTGGGN
361 TCCTGGCCAA GCCCACNANT CTAANNAACN CNTCGATGTT CCGGGGTGCC CGTGTGGATT
421 TGAAGTGGAA GTTCTGCATN ATGTNNGTNA AGGAGNNAAG AATTCCCTCC TAACCAGTNC
481 TTCTNCCAAA CCATACCGNN TCCCATNGAA AANGGCCNAA AGNCNTCATT CTTCCNNAAN
541 NGGTTCCCNN TCAATTTAAG GAAGTGCCTT NGGGTNGAAT TTTTTGGNGT NGGAAAANAC
601 CTGGGGGTCT TTCACCAAAA AACCCCACNN AAGGANCCCN TTAATAACCC TGGGGGAGAA
661 GGAATTTCCA AAACTNGGGT CCTGNNTGAC CCTNAANCCC GCCCCAGGGN ATTGGNTTTA
721 NNTACCTTGC TCCTNNNAAC TCAAGGCGGA ATGCT
```

B 38.2: Sequencing alignment of cDNA subclone **AC4#3** with **mouse Cyp2a5** by BLAST searching against the National Center for Biotechnology Information database

```
AC4#3          : 116  gccataaataatatctacttttattattttattatccctatagacaacatagtataaatt 175
                      ||||||||||||||||||||||||||||||||||||||| ||||| ||||||||||||||||||
Mouse Cyp2a5: 1688 gccataaataatatctacttttattattttattattcctattgacaacatagtataaatt 1629

AC4#3          : 176  tccctcttctttggctacctttgaaatatcctctgcaagtctactctgtgtncctctnnt 235
                      |||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||  |
Mouse Cyp2a5: 1628 tccctcttctttggctacctttgaaatatcctctgcaagtctactctgtgt-cctctgtt 1570

AC4#3          : 236  tcttctgtacctttgacctttttctaagtttcaacccctgnacttggtctgnnctcaatca 295
                      |||||||||||||||||||||||||||||||||||||||||| |||| ||||| ||||||||
Mouse Cyp2a5: 1569 tcttctgtacctttgacctttttctaagtttcaacccctg-actt-gtctggtctcaatca 1512

AC4#3          : 296  ttccctttaacctcatgcagcccaggntcaacgggacnagaaactcntagtgtaggttgg 355
                      |||||||||||||||||||||||||| |||||||||| |||||||| ||||||||||||||
Mouse Cyp2a5: 1511 ttccctttaacctcatgcagcccaggctcaacgggacaagaaactcatagtgtaggttgg 1452

AC4#3          : 356  tgggntcctggccaagcccacnantctaannaacncntcgatgttccggggtgcccgtgt 415
                      |||| || |||| |||||||| | ||||    || | ||||||| | |||||||| ||||
Mouse Cyp2a5: 1451 tgggatcgtggcaaagcccacgagtctaggagacacatcgatgtcctggggtgcctgtgt 1392

AC4#3          : 416  ggatttgaagtggaagttctgcatnatgt 444
                      |||||||||||||||||||||||| ||||
Mouse Cyp2a5: 1391 ggatttgaagtggaagttctgcatgatgt 1363
```

B 38.3: Summary of sequence alignment of cDNA subclone **AC4#3** with **mouse Cyp2a5**

FluoroDD gel AC (AP2 + ARP19)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AC4	++	+	++	++	1100	AC4#3	1050	M13 forward (-20)	*Mus musculus* cytochrome P450, family 2, subfamily a, polypeptide 5 (Cyp2a5) (1720 bp)	e-121 302/329 (91%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 39.1: DNA sequence of cDNA subclone AC4#3 (AP2 & ARP19) using M13 reverse primer

```
  1 CNCCNGANCA TTTAGGTGAC CTATAGAATA CTCAAGCTAT GCATCAAGCN TTGGTACGAC
 61 TCGGATCCAN NTAGTANGGC CNCCAAGTGT GCATGGNAAN NGCCCTTACN GGNTACAATT   ARP19
121 TCACACAGGA TTTTGGCTCC CACTGCTTAA ATACAGACTA GGACAGGGCT CTGTCTCCTC
181 AGCCTCGGTC ACCACCCAGC TCTGGGACAG CNAAGCTGAA AATGACTCCC TCCATCTCAT
241 GGGGTCTACT GCTTCTGGCA GGCCTGTGTT GCCTGGTCCC CANGCNTTTC TGGCTGAGGA
301 TGTTCAGGAN ACAGACACCT CCCNAGAAGG ATCCAGCCCC CAGCCTCCCA TGAGATCGCT
361 ACAAACCTGG GNANACTTTG CAATCNAGCC TATACCGGGN AGCNGGGTCC ATCCAGNNCC
421 AACACCNTCC CAACATNCCC TCCTTCCTCC CCCCAGGTGG AGNCANTTTG NCCCACCAGC
481 CCNNNTTTGN CNTTATTGNC CTCCCTCCCC CNTNAGNGGG NAGNCCAAAG GGGNNTTGNA
541 CAANCCTTCC ACCANCCGGC CCAAGAATCC CCCCCCCATT GGGGGGCCCC CTTGGGGCNC
601 TTTCGGAAAN NGGNNNTNCC ACCCCCAAAA GGGGGACCAA CNNCAAAAAG GTTTTTCCGN
661 NAAAGNAAAT TTTTTTNNCC TTTTCCCCTC CCCCCCCCCC AAAAAGNNGG GNTNNANNAT
721 TTGGGAAAAN GTTGGGGTTT TTTCCNNTCN AATNTGCGNN TTGGGGGGGC CTTTTTTTGN
781 TGTTTGNNNN TTGGNAAAAA AAGANAACNN CNNCCCCCAA AAAGGTTNTT TTTNTNTTTT
841 TTTNNNNNNN NCNNNNNNNN
```

B 39.2: Sequencing alignment of cDNA subclone **AC4#3** with **mouse serine (or cysteine) proteinase inhibitor (SPI)** by BLAST searching against the National Center for Biotechnology Information database

```
AC4#3     : 171 ctgtctcctcagcctcggtcaccacccagctctgggacagcnaagctgaaaatgactccc 230
                ||||||||||||||||||||||||||||||||||||||||||| |||||||||||||||||
Mouse SPI: 1   ctgtctcctcagcctcggtcaccacccagctctgggacagc-aagctgaaaatgactccc 59

AC4#3     : 231 tccatctcatggggtctactgcttctggcaggcctgtgttgcctggtccccangcntttc 290
                ||||||||||||||||||||||||||||||||||||||||||||||||||||| || ||||
Mouse SPI: 60  tccatctcatggggtctactgcttctggcaggcctgtgttgcctggtcccca-gc-tttc 117

AC4#3     : 291 tggctgaggatgttcaggagacagacacctcccnagaaggatccagcccccagcctccca 350
                |||||||||||||||||| |||||||||||| |||||||| ||| ||||||||||||||
Mouse SPI: 118 tggctgaggatgttcaggagacagacacctccc-agaaggat-cagtccccagcctccca 175

AC4#3     : 351 tgagatcgctacaaacctgggnanactttgcaatcnagcctataccggg 399
                |||||||||||||||||||| | |||||||||||| ||||||||||||||
Mouse SPI: 176 tgagatcgctacaaacctggg-agactttgcaatc-agcctataccggg 222
```

B 39.3: Summary of sequence alignment of cDNA subclone **AC4#3** with **mouse SPI**

FluoroDD gel AC (AP2 + ARP19)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AC4	++	+	++	++	1100	AC4#3	1050	M13 reverse	*Mus musculus* serine (or cysteine) proteinase inhibitor clade A, member 1a (SPI) (1393 bp)	4e-85 219/229 (95%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 40.1: DNA sequence of cDNA subclone AC7#5 (AP2& ARP19) using M13 forward (-20) primer

```
  1 AACNCTCACG TATAGGGCGA ATTGGGCCCT CTAGATGCAT GCTCGAGACG GCCGCCAGTG
 61 TGATGGATAT CTGCAGAATT CGCCCTTGTA ATACGACTCA CTATAGGGCT TTTTTTTTTT   AP2
121 TGCTGTAGCA TCAGGCACGA NGNCCAGGCC AGCCTGTGTT TAATGGAAGG AACCCAATTC
181 AGAAGGAAGG ATGACATTTT AGGGTGGTCA CTTTATGTGT GGGATCTACC ACTTTTCCCA
241 CAAAGATGGG GCTCTGAGTG TGTTCTTCAA ATATTATGAA AAGGAAAGGG TGGTCGAAGC
301 GCAGGATAGG GGGCATAAGA CATAGGAACC ATTTGTAAGA CTGTAACTGC TGCAGCTTCT
361 GTTCCTGTCT CATCGATGGT CAGCACAGCC TTATGCACAG CCTGGCTGAG CTTCAGGGGA
421 GCATTCTCCC TCTGTGATTC CGGAGAGGTC AGCCCCATTG TTGAAAATCC NGGGTGATGC
481 CCAGTGGACT CATGAGTGTN CTTCNANGTT ATATTCTCCA AAGATGGGAC AGTNNGGGNG
541 AAGTGGATTT GGNGCTAACT GGTGGGTNGG TAANANTNTN NTNNGAGTNN TGGGGATNNG
601 GNTTTGNTNA NNNNGTNGAN TNNTNNNNTG GNTCTNNNTG TNNTNGNNTN NNNNNNNNGN
661 NNNNGNNNNN NNTTNN
```

B 40.2: Sequencing alignment of cDNA subclone **AC7#5** with **mouse serine (or cysteine) proteinase inhibitor (SPI)** by BLAST searching against the National Center for Biotechnology Information database

```
AC7#5     : 122  gctgtagcatcaggcacgangnccaggccagcctgtgtttaatggaaggaacccaattca 181
                 ||||||||||||||||||   ||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1376 gctgtagcatcaggcacgag--ccaggccagcctgtgtttaatggaaggaacccaattca 1319

AC7#5     : 182  gaaggaaggatgacattttagggtggtcactttatgtgtgggatctaccacttttcccac 241
                 ||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Mouse SPI: 1318 gaaggaaggatgacattttagggtggtca-tttatgtgtgggatctaccacttttcccac 1260

AC7#5     : 242  aaagatggggctctgagtgtgttcttcaaatattatgaaaaggaaagggtggtcgaagcg 301
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1259 aaagatggggctctgagtgtgttcttcaaatattatgaaaaggaaagggtggtcgaagcg 1200

AC7#5     : 302  caggataggggcataagacataggaaccatttgtaagactgtaactgctgcagcttctg 361
                 |||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1199 caggataggggcata-gacataggaaccatttgtaagactgtaactgctgcagcttctg 1141

AC7#5     : 362  ttcctgtctcatcgatggtcagcacagccttatgcacagcctggctgagcttcaggggag 421
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1140 ttcctgtctcatcgatggtcagcacagccttatgcacagcctggctgagcttcaggggag 1081

AC7#5     : 422  cattctccctctgtgattccggagaggtcagccccattgttgaaaatccngggtgatgcc 481
                 ||||||  ||||||||||||||||||||||||||||||||||||  ||||  ||||||||||
Mouse SPI: 1080 cattct-cctctgtgattccggagaggtcagccccattgttgaagatcc-gggtgatgcc 1023

AC7#5     : 482  cagtggactcatgagtgtncttcnangttatattctccaaagatgg 527
                 ||||||||||||||||| ||||  | ||||||||||||  ||||||
Mouse SPI: 1022 cagtggactcatgagtgt-cttc-aagttatattctccagagatgg 979
```

B 40.3: Summary of sequence alignment of cDNA subclone **AC7#5** with **mouse SPI**

FluoroDD gel AC (AP2 + ARP19)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AC7	+	-	+	+	770	AC7#5	790	M13 forward (-20)	*Mus musculus* serine (or cysteine) proteinase inhibitor clade A, member 1a (SPI) (1393 bp)	0 394/406 (97%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 41.1: DNA sequence of cDNA subclone AD6#4 (AP2 & ARP18) using M13 reverse primer

```
  1 AGACTATTTA GGTGACACTA TAGAATACTA CNAGACTATG ACNTNCAAGA CTTGGTACCG
 61 AGACTCGGAT CNCTAGTANN GGCCGCCAGT GTGCTGGAAT TCGCCCTTAG ACGGATACAA   ARP18
121 TTTCACACAG GATGATGCTA CCACTTGTCA CATTGTAGTC CTGAGACATA CAGGAAATGG
181 GGCAACCTGC CTGACACATT GTGATGGATC TGACACCAAA GCTGAAGTCC CCTTGATAAT
241 GAGCTCCATA AAATCCTTCT CTGAACATGC TGAGTGTGGA AGGCTGGAAG TACATCTTGT
301 GGGAGGCTTC AGTGATGACA GGCAGCTGTC ACAGAAACTT ACTCATCAGC TTCTCAGTGA
361 ATTTGACAAA CAGGATGATG ACATTCACTT AGTGACATTA TGTGTAACAG AGTTAAATGA
421 CCGTGAAGAN AAATGAAAAT CACNTTTCCA ATCATTTATG GCATCGCCTG TCAACATNAA
481 AACTGCCAGA GATTTACAGA GCTNCCCTTT CAAGATCGTG GTCCCAGAGG AGCAACTGCG
541 TGCTGCAAGA GCTTTAGCAC GANGCCCAAT GATTAGCATT TATGATGCCA NGACAGNNCA
601 CCTCCGANNA GGCNCGTGTT CCTGNACCNN ATTCCCCACA AAGTGGATCN TCTGGGTTGC
661 CGCAAAGAAT GACNNNGCCN ANTTTCNTAA NANAAGNCCT TTTCCNACGN TNNTTCCTCT
721 GGNNCCNCGC CCCCCCCCCC CTNTNGGTTG AANTCTTAAA CTCCTNNCCC TGATTNTTNN
781 TNNNTNNNTT TTTNNNNNNN TTTCTCTTNT TTGGAATTTC CCCCNCCNNN NCCNNNCTNT
841 NNNTGNTGTN GTGTNNNNNN NTNNTTCGG
```

B 41.2: Sequencing alignment of cDNA subclone **AD6#4** with **mouse N-terminal Asn amidase (Ntanl)** by BLAST searching against the National Center for Biotechnology Information database

```
AD6#4     : 131 gatgatgctaccacttgtcacattgtagtcctgagacatacaggaaatggggcaacctgc 190
Mouse Asn: 285 gatgatgctaccacttgtcacattgtagtcctgagacatacaggaaatggggcaacctgc 344

AD6#4     : 191 ctgacacattgtgatggatctgacaccaaagctgaagtccccttgataatgagctccata 250
Mouse Asn: 345 ctgacacattgtgatggatctgacaccaaagctgaagtccccttgataatgagctccata 404

AD6#4     : 251 aaatccttctctgaacatgctgagtgtggaaggctggaagtacatcttgtgggaggcttc 310
Mouse Asn: 405 aaatccttctctgaacatgctgagtgtggaaggctggaagtacatcttgtgggaggcttc 464

AD6#4     : 311 agtgatgacaggcagctgtcacagaaacttactcatcagcttctcagtgaatttgacaaa 370
Mouse Asn: 465 agtgatgacaggcagctgtcacagaaacttactcatcagcttctcagtgaatttgacaaa 524

AD6#4     : 371 caggatgatgacattcacttagtgacattatgtgtaacagagttaaatgaccgtgaagan 430
Mouse Asn: 525 caggatgatgacattcacttagtgacattatgtgtaacagagttaaatgaccgtgaaga- 583

AD6#4     : 431 aaatgaaaatcacntttccaatcatttatggcatcgcctgtcaacatnaaaactgccaga 490
Mouse Asn: 584 aaatgaaaatcac-tttccaatcatttatggcatcg-ctgtcaacattaaaactg-caga 640

AD6#4     : 491 gatttacagagctnccctttcaagatcgtggtcccagaggagcaactgcgtgctgcaaga 550
Mouse Asn: 641 gatttacagagctt-cctttcaagatcgtggt-ccagaggagcaactgcgtgctgcaaga 698

AD6#4     : 551 gctttagcacgangcccaatgattagcatttatgatgccangacagnncacctccganna 610
Mouse Asn: 699 gctttagcaggaggcccaatgattagcatttatgatgcaaagacagaacaactccgaata 758

AD6#4     : 611 ggcncgtgttcctg 624
Mouse Asn: 759 ggcccgtgttcctg 772
```

B 41.3: Summary of sequence alignment of cDNA subclone **AD6#4** with **mouse Ntanl**

FluoroDD gel AD (AP2 + ARP18)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AD6	+	++	+	+	840	AD6#4	860	M13 reverse	*Mus musculus* N-terminal Asn amidase (Ntanl) (1184 bp)	0 476/494 (96%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 42.1: DNA sequence of cDNA subclone AD6#10 (AP2 & ARP18) using M13 forward (-20) primer

```
  1 AACNNAACCN NTANNGGCGA ATNGGGCCCT ACTAGATGCT GACTCAGACN NCCGNCACGN
 61 TNNGAATNGA NTNTCNGACG AANTTCGACC CTGANNANNT ACGACTCACT ATAGGGCTTT   AP2
121 TTTTTTTTTG CATGAACACT TGGAACCTTT ATTGNCAGAA AGGAGGGAAG ATGGAGAAAA
181 CTTGTGTGAG GATTAATAGG GTAGAGAGAG ACTATGACAG GGTAGATTTA GATGAACANA
241 TTAGGTAATT GTCATGTGAA AATGATACTA GGCTGAATAA TGGCTTCACA ATGACATTNC
301 AGGCCTTGGG TTTGGAGNCC CATGGTACAT ATTGAATAAT TCCTCATAAT TCAAAAGGAA
361 CTTAGTAGGT TAGATTGAAT AAGATATATT TAGATGAAGA AAATATCTTG GAGCTTTTAG
421 GGATCTTGAT GATGTCATAA GCATTTGTAA AAGGGAAGGA GATTAGAAGA GACAGGACGA
481 GGAGGGTGGT GGCATTGAAG CAGGGGAATA TTCCAGTGCC CAAGGAATGA TGATCTATAC
541 TGAACAGAGG ATGAGAGGAC TCCTCTGGAG TACTCTTCTG AAGAANGTNG GTCACACCAA
601 CAACTTAGNN TTCTGCCAGT GANACTCATC TCAGACNTGG AGCTCCACAA CTGTAAGATA
661 ACCACTNCAA ATTATNCAAA NNNCCANGGT GNGACAATGA TATTGTAAAT GANNAGNNTG
721 TNNGAAAATA GATGTAGAGT GAAGCANGAA ATGNNGAGAG NNANGTTTAT NGTTNGTNNN
781 NTNGTTAATG TNCGGTGGCN NTCNNNNNNG CGTNTNANNG
```

B 42.2: Sequencing alignment of cDNA subclone **AD6#10** with **mouse Cyp4a10** by BLAST searching against the National Center for Biotechnology Information database

```
AD6#10          : 147  ctttattgncagaaaggagggaagatggagaaaacttgtgtgaggattaatagggtagag 206
                       ||||||||| ||||||||||||||||||||||||| || |||||||||||||  |||||||
Mouse Cyp4a10: 2054 ctttattg-cagaaaggagggaagatggagaaa-ctcgtgtgaggattaatcaggtagaa 1997

AD6#10          : 207  agagactatgacagggtagatttagatgaacanattaggtaattgtcatgtgaaaatgat 266
                       |||||||||| |||||||||||||||||||||| |||||||| ||||||||||||||||||
Mouse Cyp4a10: 1996 agagactatg-cagggtagatttagatgaacacattaggtaactgtcatgtgaaaatgat 1938

AD6#10          : 267  actaggctgaataatggcttcacaatgacattncaggccttgggttt-ggagncccatgg 325
                       |||| |||||||||| |||||||||| || |||||||||| ||| |||| || ||||
Mouse Cyp4a10: 1937 actaagctgaataatgtcttcacaatgatgtt-caggccttggattttggagacc-atgg 1880

AD6#10          : 326  tacatattgaataattcctcataattcaaaaggaacttagtaggttagattgaataagat 385
                       |||||||||||| |||||||||||||| ||||||||||||||||||||||||||||||||
Mouse Cyp4a10: 1879 tacatattgaatgattcctcataattcagaaggaacttagtaggttagattgaataagat 1820

AD6#10          : 386  atatttagatgaagaaaatatcttggagcttttagggatcttgatgatgtcataagcatt 445
                       | |||||||||   ||| ||||||||||||||||||||||| ||||||||||||||||
Mouse Cyp4a10: 1819 acatttagatgggaaaactatcttggagcttttagggatcttaatgatgtcataagcatc 1760

AD6#10          : 446  tgtaaaagggaaggagattagaagagacaggacgaggagggtggtggcattgaagcaggg 505
                       ||||||||| ||||| |||||||||| ||| |||||||||| |||| ||||||||||
Mouse Cyp4a10: 1759 tgtaaaaggtaaggacattagaagagaggggatgaggagggtgctggctttgaagcaggg 1700

AD6#10          : 506  gaatattccagtgcccaaggaatgatgatctatactgaacagaggatgagaggactcctc 565
                       |||||||||||||| ||||||||||||||||||||||||||||||||||||||||| |||
Mouse Cyp4a10: 1699 gaatattccagtg-ccaaggaatgatgatctatactgaacagaggatgagaggact-ctc 1642

AD6#10          : 566  tggagtactcttctgaagaangtnggtcacaccaacaacttagnnttctgccagtganac 625
                       ||||||| ||||||||| || || |||||||||||||||||| |||| ||||||| ||
Mouse Cyp4a10: 1641 tggagtattcttctgaaaaaggtaggtcacaccaacaacttagctttct-ccagtgaaac 1583

AD6#10          : 626  tcatctcagac-ntggagctccacaac 651
                       || |||||||| |||||||||||||||
Mouse Cyp4a10: 1582 tcttctcagacattggagctccacaac 1556
```

B 42.3: Summary of sequence alignment of cDNA subclone **AD6#10** with **mouse Cyp4a10**

FluoroDD gel AD (AP2 + ARP18)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AD6	+	++	+	+	840	AD6#10	850	M13 forward (-20)	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 10 (Cyp4a10) (2085 bp)	e-159 460/507 (90%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 43.1: DNA sequence of cDNA subclone AD6#10 (AP2 & ARP18) using M13 reverse primer

```
  1 AGCCTATTTA GGTGACACTA TAGAATACTC AAGCTATGCA TCAAGCTTGG TACCGAGCTC
 61 GGATCCACTA GTAACGGCCG CCAGTGTGCT GGAATTCGCC CTTAGCGGAT AACAATTTCA   ARP18
121 CACAGGATGA TGCTACCTAT ACCTGATCAT GAACCTTCTC CCTCCTCCAG CCATCTCTCC
181 TTGCCTCTCA CTTCCTACCA TCTTATTTTT CCCTACATGA CTTCTTCATT TTACAAATAA
241 TCAATTGTCA CAACCTTGGT GGTCTGAAAT AATTGAAAGT TGTTATCTTA CAGTTGTGGA
301 GCTCCATGTC TGAGATGAGT TTCACTGGCA GAAAGCTAAG TTGTTGGTGT GACCAACCTT
361 CTTCAGAAGA GTACTCCAGA GAGTCCTCTC ATCCTCTGTT CAGTATAGAT CATCATTCCT
421 TGGCACTGGA ATATTCCCCT GCTTCAATGC CACCACCCTC CTCGTCCTGT CTCTTCTAAT
481 CTCCTTCCCT TTTACAAATG CTTATGACAT CATCAAGATC CCTAAAAGCT CCAAGATATT
541 TTCTTCATCT AAATATATCT TATTCAATCT AACCTACTAA GTNCCTTTTG AATTATGAGG
601 AATTATTCAA TATGTACCAT GGGCTCCAAA CCCCAAGGCC TGAATGTCAT AGNCACCCCA
661 TAATACAGCC TAGTATCATT TCCACATGAC AATNNNCTAA TGTGTNCATC TAAATCTACC
721 CTGCATAGTC TCTCTCTACC CTATTAATCC TCACACAAGT NNCTCATCNT CCCTCCTTCT
781 GCANNAAAGG TCNNNGTGNT ATGCAAAAAA ANNANNCCCN NATAGTGAA
```

B 43.2: Sequencing alignment of cDNA subclone **AD6#10** with **mouse Cyp4a10** by BLAST searching against the National Center for Biotechnology Information database

```
AD6#10        : 293  gttgtggagctcca-tgtctgagatgagtttcactggcagaaagctaagttgttggtgtg 351
Mouse Cyp4a10: 1556 gttgtggagctccaatgtctgagaagagtttcactgg-agaaagctaagttgttggtgtg 1614

AD6#10        : 352  accaaccttcttcagaagagtactccagagagtcctctcatcctctgttcagtatagatc 411
Mouse Cyp4a10: 1615 acctacctttttcagaagaatactccagagagtcctctcatcctctgttcagtatagatc 1674

AD6#10        : 412  atcattccttggcactggaatattcccctgcttcaatgccaccaccctcctcgtcctgtc 471
Mouse Cyp4a10: 1675 atcattccttggcactggaatattcccctgcttcaaagccagcaccctcctcatccctc 1734

AD6#10        : 472  tcttctaatctccttccctttacaaatgcttatgacatcatcaagatccctaaaagctc 531
Mouse Cyp4a10: 1735 tcttctaatgtccttacctttacagatgcttatgacatcattaagatccctaaaagctc 1794

AD6#10        : 532  caagatattttcttcatctaaatatatcttattcaatctaacctactaagtncctttga 591
Mouse Cyp4a10: 1795 caagatagttttcccatctaaatgtatcttattcaatctaacctactaagttccttctga 1854

AD6#10        : 592  attatgaggaattattcaatatgtaccatgggctccaaaccccaaggcctgaatgtcata 651
Mouse Cyp4a10: 1855 attatgaggaatcattcaatatgtaccatggtctccaaaatccaaggcctgaacatcatt 1914

AD6#10        : 652  gncaccccataatacagcctagtatcatttccacatgacaatnnnctaatgtgtncatct 711
Mouse Cyp4a10: 1915 gtgaagacattattcagcttagtatcattttcacatgacagttacctaatgtgttcatct 1974

AD6#10        : 712  aaatctaccctgcatagtctctctctaccctattaatcctcacacaagtnnct-catcnt 770
Mouse Cyp4a10: 1975 aaatctaccctgcatagtctctttctacctgattaatcctcacacgagtttctccatctt 2034

AD6#10        : 771  ccctcctt 778
Mouse Cyp4a10: 2035 ccctcctt 2042
```

B 43.3: Summary of sequence alignment of cDNA subclone **AD6#10** with **mouse Cyp4a10**

FluoroDD gel AD (AP2 + ARP18)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AD6	+	++	+	+	840	AD6#10	850	M13 reverse	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 10 (Cyp4a10) (2085 bp)	e-155 437/488 (89%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 44.1: DNA sequence of cDNA subclone AD8#2 (AP2 & ARP18) using M13 forward (-20) primer

```
  1 TGGGCCCTCT AGATGCATGC TCGAGCGGCC GCCAGTGTGA TGGATATCTG CAGAATTCGC
 61 CCTTGTAATA CGACTCACTA TAGGGCTTTT TTTTTGCATG ACACTTGGAC CTTTATTGCA   AP2*
121 GAAGGGAGGG AAGATGGAGA AACTTGTGTG AGGATTAATA GGGTAGAGAG AGACTATGCA
181 GGGTAGATTT AGATGAACAC ATTAGGTAAT TGTCATGTGA AAATGATACT AGGCTGAATA
241 ATGGCTTCAC AATGACATTC AGGCCTTGGG TTTGGAGCCC ATGGTACATA TTGAATAATT
301 CCTCATAATT CAAAAGGAAC TTAGTAGGTN AGATTGAATA AGATATATTT AGATGAAGAA
361 AATATCTTGG AGCTTTTAGG GATCTTGATG ATGTCATAAG CATNTGTAAA AGGGAAGGAG
421 ATNAGAAGAG ACAGGACGAG GAGGGTGGTG GCATNGAAGC AGGGGAATAT TCCAGTGCCA
481 AGGAATGATG ATCTATACTG AACAGAGGAT GAGAGGACTC TCTGGAGTAC TCTTCTGAAG
541 AAGGTTGGTC ACACCAACAA CTTAGCTTTC TGCCAGTGAN ACTCATCTCA GACATGGAGC
601 TCCACAACTG TAAGATAACA NCTTTCAATT ATNNCAACCA CCAGGGTGTG ACANTGATAT
661 TGTNAATAGA GTNGTNGGAA ATAGATGTAG AGGAAGCAGA AATCNGAGAG NAAGTATATC
721 GTTNGTCCTN TCGGTANGNN CCAGNCA
```

B 44.2: Sequencing alignment of cDNA subclone **AD8#2**with **mouse Cyp4a10** by BLAST searching against the National Center for Biotechnology Information database

```
AD8#2          : 96   gcatgacacttggacctttattgcagaagggagggaagatggagaaacttgtgtgaggat 155
                      ||||||||||| ||| |||||||||||||| ||||||||||||||||||||| ||||||||||
Mouse Cyp4a10: 2069 gcatgacactgggaactttattgcagaaaggagggaagatggagaaactcgtgtgaggat 2010

AD8#2          : 156  taatagggtagagagagactatgcagggtagatttagatgaacacattaggtaattgtca 215
                      ||||  |||||| ||||||||||||||||||||||||||||||||||||||||||| |||||
Mouse Cyp4a10: 2009 taatcaggtagaaagagactatgcagggtagatttagatgaacacattaggtaactgtca 1950

AD8#2          : 216  tgtgaaaatgatactaggctgaataatggcttcacaatgacattcaggccttgggttt-g 274
                      |||||||||||||||| |||||||||| |||||||||| |||||||||||| ||| |
Mouse Cyp4a10: 1949 tgtgaaaatgatactaagctgaataatgtcttcacaatgatgttcaggccttggattttg 1890

AD8#2          : 275  gagcccatggtacatattgaataattcctcataattcaaaaggaacttagtaggtnagat 334
                      ||| |||||||||||||||| |||||||||||||| |||||||||||||| ||||
Mouse Cyp4a10: 1889 gagaccatggtacatattgaatgattcctcataattcagaaggaacttagtaggttagat 1830

AD8#2          : 335  tgaataagatatatttagatgaagaaaatatcttggagcttttagggatcttgatgatgt 394
                      |||||||||| ||||||||   ||| |||||||||||||||||||||| |||||||
Mouse Cyp4a10: 1829 tgaataagatacatttagatgggaaaactatcttggagcttttagggatcttaatgatgt 1770

AD8#2          : 395  cataagcatntgtaaaagggaaggagatnagaagagacaggacgaggagggtggtggcat 454
                      |||||||||| |||||||| |||||| || |||||||| ||| |||||||||| |||| |
Mouse Cyp4a10: 1769 cataagcatctgtaaaaggtaaggacattagaagagaggggatgaggagggtgctggctt 1710

AD8#2          : 455  ngaagcaggggaatattccagtgccaaggaatgatgatctatactgaacagaggatgaga 514
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a10: 1709 tgaagcaggggaatattccagtgccaaggaatgatgatctatactgaacagaggatgaga 1650

AD8#2          : 515  ggactctctggagtactcttctgaagaaggttggtcacaccaacaacttagctttctgcc 574
                      |||||||||||||||| ||||||||| ||||| ||||||||||||||||||||||| ||
Mouse Cyp4a10: 1649 ggactctctggagtattcttctgaaaaaggtaggtcacaccaacaacttagctttct-cc 1591

AD8#2          : 575  agtganactcatctcagaca-tggagctccacaac 608
                      ||||| |||| ||||||||| ||||||||||||||
Mouse Cyp4a10: 1590 agtgaaactcttctcagacattggagctccacaac 1556
```

B 44.3: Summary of sequence alignment of cDNA subclone **AD8#2** with **mouse Cyp4a10**

FluoroDD gel AD (AP2 + ARP18)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AD8	+	++	+	+	720	AD8#2	780	M13 forward (-20)	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 10 (Cyp4a10) (2085 bp)	0 474/515 (92%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 45.1: DNA sequence of cDNA subclone AD8#7 (AP2 & ARP18) using M13 reverse primer

```
  1 ACTATTTAGG TGACACTATA GAATACTACA AGACTATGNC ATNCAAGACT TGGTACCGAG
 61 ACTCGGATCN ACTAGTAACG GCCGCCAGTG TGCTGGAATT CGCCCTTAGC GGATAACAAT   ARP18
121 TTCACACAGG ATGATGCTAC CTATACCTGA TCATGAACCT TCTCCCTCCT CCAGCCATCT
181 CTCCTTGCCT CTCACTTCCT ACCATCTTAT TTTTCCCTAC ATGACTTCTT CATTTTACAA
241 ATAATCAATT GTCACAACCT TGGCGGTCTG AAATAATTGA AAGTTGTTAT CTTACAGTTG
301 TGGAGCTCCA TGTCTGAGAT GAGTTTCACT GGCAGAAAGC TAAGTTGTTG GTGTGACCAA
361 CCTTCTTCAG AAGAGTACTC CAGAGAGTCC TCTCATCCTC TGTTCAGTAT AGATCNATCA
421 TTCCTTGGCA CTGGAATATT CCCCCTGCTT CAATGCCACC ACCCTCCCTC GTCCTGTCTC
481 TTCTAATCTC CTTCCCCTTT TACAAATGCT TATGACATCA TCAAGNATCC CTAAAAGCTC
541 CAAGATATTT TCTTCATCTA AATATATCTT ANNCAATCTA ACCTACTAAG TTCCTTTTGA
601 NTTATGANGA ATTATTCAAT ATGTACCATG GGCTCCAAAC CCAAGGGCCT GAATGTCATT
661 GTGANGCCAT TATCCAGCCC TAGTATCATT TNNACANTGA CAAATTACCC TAANTGTGNT
721 TCATCTAAAN NNCTACCCNT GCCATAGNTT TTCTNTTCTT ACNCCNTANT NNANTTTNCN
781 TCACANNNAN GTTNNTCCTC CNANTNTTNC CCNNTNCNTT TTTCNTGCAA
```

B 45.2: Sequencing alignment of cDNA subclone **AD8#7** with **mouse Cyp4a10** by BLAST searching against the National Center for Biotechnology Information database

```
AD8#7          : 297  gttgtggagctcca-tgtctgagatgagtttcactggcagaaagctaagttgttggtgtg 355
                      ||||||||||||||| |||||||| |||||||||||| ||||||||||||||||||||||
Mouse Cyp4a10: 1556 gttgtggagctccaatgtctgagaagagtttcactgg-agaaagctaagttgttggtgtg 1614

AD8#7          : 356  accaaccttcttcagaagagtactccagagagtcctctcatcctctgttcagtatagatc 415
                      ||| |||||| |||||||| ||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a10: 1615 acctacctttttcagaagaatactccagagagtcctctcatcctctgttcagtatagatc 1674

AD8#7          : 416  natcattccttggcactggaatattccccctgcttcaatgccaccaccctccctcgtcct 475
                      |||||||||||||||||||||||||||||||||||| |||||||| |||| |||||||| || |||
Mouse Cyp4a10: 1675 -atcattccttggcactggaatattcccc-tgcttcaaagccagcaccctcc-tcatccc 1731

AD8#7          : 476  gtctcttctaatctccttcccctttacaaatgcttatgacatcatcaagnatccctaaa 535
                      |||||||||||| ||||| || |||||||| |||||||||||||||| ||| ||||||||||
Mouse Cyp4a10: 1732 ctctcttctaatgtccttacc-ttttacagatgcttatgacatcattaag-atccctaaa 1789

AD8#7          : 536  agctccaagatattttcttcatctaaatatatcttanncaatctaacctactaagttcct 595
                      |||||||||||| |||   ||||||||| ||||||| ||||||||||||||||||||||||
Mouse Cyp4a10: 1790 agctccaagatagttttcccatctaaatgtatcttattcaatctaacctactaagttcct 1849

AD8#7          : 596  tttganttatgangaattattcaatatgtaccatgggctccaaacccaagggcctgaatg 655
                      | ||| |||||| |||| ||||||||||||||||||| |||||||  | | |||||||||
Mouse Cyp4a10: 1850 tctgaattatgaggaatcattcaatatgtaccatggtctccaaaatccaaggcctgaaca 1909

AD8#7          : 656  tcattgtgangccattatccagccctagtatcattt 691
                      ||||||||| | |||||| ||| | ||||||||||||
Mouse Cyp4a10: 1910 tcattgtgaagacattattcag-cttagtatcattt 1944
```

B 45.3: Summary of sequence alignment of cDNA subclone **AD8#7** with **mouse Cyp4a10**

FluoroDD gel AD (AP2 + ARP18)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AD8	+	++	+	+	720	AD8#7	780	M13 reverse	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 10 (Cyp4a10) (2085 bp)	e-105 353/396 (89%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 46.1: DNA sequence of cDNA subclone AD9#2 (AP2 & ARP18) using M13 forward (-20) primer

```
  1 CCCTCACTAT AGGGCGAATT GGGCCCTCTA GATGCNTGCT CGAGCGGCCG CCAGTGTGAT
 61 GGATNTCTGC AGAATTCGCC CTTGTAATAC GACTCACTAT AGGGCTTTTT TTTTTTGCAT   AP2*
121 GACACTGGGA ACTTTATTGC AGAAAGGAGG GAAGATGGAG AAACTCGTGT GAGGATTAAT
181 CAGGTAGAAA GAGACTATGC AGGGTAGATT TAGATGAACA CATTAGGTAA TTGTCATGTG
241 AAAATGATAC TAGGCTGAAT AATGGCTTCA CAATGACATT CAGGCCTTGG GTTTGGAGCC
301 CATGGTACAT ATTGAATAAT TCCTCATAAT TCAAAAGGAA CTTAGTAGGT TAGATTGAAT
361 AAGATATATT TAGATGAAGA AAATATCTTG GAGCTTTTAG GGATCTTGAT GATGTCATAA
421 GCATTTGTAA AAGGGAAGGA GATTAGAAGA GACAGGACGA GGAGGNNGGT GGCATTGACG
481 CAGGGGAATA NNCCAGTGCC AAGGAATGAT GATCTATACT GAACAGAGGA TGAGAGGACT
541 CTCTGGAGTA CTCTTCTGAA GAGGGTNGGT CACACCACCA ACTTAGNNTT NTGCCAGTGA
601 NACTCATCTC AGACATGGAG CTCCACACTG TAAGATACNC CTTTTNATAA TNTAAANCAN
661 CNANNTGTGA CAANNATTAT NNGTAAATGA AANNTTTGTA NNNANNATAG ATGNTAGANT
721 TAAAGCCAGG AAAATNNTGA AGAGGNNNAG TCNTNATNGT TTNGTNTTTN GGGTANTTTT
```

B 46.2: Sequencing alignment of cDNA subclone **AD9#2** with **mouse Cyp4a10** by BLAST searching against the National Center for Biotechnology Information database

```
AD9#2          : 117  gcatgacactgggaactttattgcagaaaggagggaagatggagaaactcgtgtgaggat 176
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a10: 2069  gcatgacactgggaactttattgcagaaaggagggaagatggagaaactcgtgtgaggat 2010

AD9#2          : 177  taatcaggtagaaagagactatgcagggtagatttagatgaacacattaggtaattgtca 236
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Mouse Cyp4a10: 2009  taatcaggtagaaagagactatgcagggtagatttagatgaacacattaggtaactgtca 1950

AD9#2          : 237  tgtgaaaatgatactaggctgaataatggcttcacaatgacattcaggccttgggttt-g 295
                      |||||||||||||||| |||||||||| |||||||||| |||||||||| ||| | 
Mouse Cyp4a10: 1949  tgtgaaaatgatactaagctgaataatgtcttcacaatgatgttcaggccttggattttg 1890

AD9#2          : 296  gagcccatggtacatattgaataattcctcataattcaaaaggaacttagtaggttagat 355
                      ||| |||||||||||||||| |||||||||||||| |||||||||||||||||||||||
Mouse Cyp4a10: 1889  gagaccatggtacatattgaatgattcctcataattcagaaggaacttagtaggttagat 1830

AD9#2          : 356  tgaataagatatatttagatgaagaaaatatcttggagcttttagggatcttgatgatgt 415
                      |||||||||| |||||||||   ||| |||||||||||||||||||||||| |||||||
Mouse Cyp4a10: 1829  tgaataagatacatttagatgggaaaactatcttggagcttttagggatcttaatgatgt 1770

AD9#2          : 416  cataagcatttgtaaaagggaaggagattagaagagacaggacgaggaggnnggtggcat 475
                      |||||||| |||||||| ||||| |||||||||| ||| ||||||| | ||||| |
Mouse Cyp4a10: 1769  cataagcatctgtaaaaggtaaggacattagaagagaggggatgaggagggtgctggctt 1710

AD9#2          : 476  tgacgcaggggaatannccagtgccaaggaatgatgatctatactgaacagaggatgaga 535
                      ||| |||||||||||  ||||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a10: 1709  tgaagcaggggaatattccagtgccaaggaatgatgatctatactgaacagaggatgaga 1650

AD9#2          : 536  ggactctctggagtactcttctgaagagggtnggtcacaccaccaacttagnnttntgcc 595
                      |||||||||||||||| ||||||||| | ||| |||||||||| |||||||| || | ||
Mouse Cyp4a10: 1649  ggactctctggagtattcttctgaaaaaggtaggtcacaccaacaacttagctttct-cc 1591

AD9#2          : 596  agtganactcatctcagaca-tggagctccaca 627
                      ||||| |||| ||||||||| ||||||||||||
Mouse Cyp4a10: 1590  agtgaaactcttctcagacattggagctccaca 1558
```

B 46.3: Summary of sequence alignment of cDNA subclone **AD9#2** with **mouse Cyp4a10**

FluoroDD gel AD (AP2 + ARP18)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AD9	+	++	+	+	710	AD9#2	765	M13 forward (-20)	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 10 (Cyp4a10) (2085 bp)	0 472/513 (92%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 47.1: DNA sequence of cDNA subclone AD9#3 (AP2 & ARP18) using M13 reverse primer

```
  1 CCNTATNTAG GTGACACTAT AGAATACTCA AGCTATGCAT CAAGCTTGGT ACCGAGCTCG
 61 GATCCACTAG TAACGGCCGC CAGTGTGCTG GAATTCGCCC TTAGCGGATA ACAATTTCAC   ARP18
121 ACAGGATGAT GCTACCTATA CCTGATCATG AACCTTCTCC CTCCTCCAGC CATCTCTCCT
181 TGCCTCTCAC TTCCTACCAT CTTATTTTTC CCTACATGAC TTCTTCATTT TACAAATAAT
241 CAATTGTCAC AACCTTGGTG GTCTGAAATA ATTGAAAGTT GTTATCTTAC AGTTGTGGAG
301 CTCCATGTCT GAGATGAGTT TCACTGGCAG AAAGCTAAGT TGTTGGTGTG ACCAACCTTC
361 TTCAGAAGAG TACTCCAGAG AGTCCTCTCA TCCTCTGTTC AGTATAGATC ATCATTCCTT
421 GGCACTGGAA TATNCCCCTG CTTCAATGCC ACCACCCTCC TCGTCCTGTC TCTTCTAATC
481 TCCTTCCCTT TTACAAATGC TTATGACATC ATCAAGATCC CTAAAAGCTC CAAGATATTT
541 TCTTCATCTA AATATATCTT ATTCAATCTA ACCTACTANG TTCCTTTTGA ATTATGAGGA
601 ATTATTCATT ATGTACCATG GGCTNCAAAN CCCAAGGCCT GAATGTCATT GTGAAGCCAT
661 TATTCAGCCT AGTATCATNN TCACATGACA ATNANNTAAT GTGTCCATCT ANATCTACCC
721 TGCATAGTCC NNCTCTANNC NATTAATCCC CCACCAAGTT TCTCCNNTCT NCCCNCNNNN
781 CTGCAAATAA NNGG
```

B 47.2: Sequencing alignment of cDNA subclone **AD9#3** with **mouse Cyp4a10** by BLAST searching against the National Center for Biotechnology Information database

```
AD9#3          : 292  gttgtggagctcca-tgtctgagatgagtttcactggcagaaagctaagttgttggtgtg 350
                      ||||||||||||||| ||||||||| |||||||||||| ||||||||||||||||||||||
Mouse Cyp4a10: 1556 gttgtggagctccaatgtctgagaagagtttcactgg-agaaagctaagttgttggtgtg 1614

AD9#3          : 351  accaaccttcttcagaagagtactccagagagtcctctcatcctctgttcagtatagatc 410
                      ||| |||||| |||||||||| ||||||||||||||||||||||||||||||||||||||||
Mouse Cyp4a10: 1615 acctacctttttcagaagaatactccagagagtcctctcatcctctgttcagtatagatc 1674

AD9#3          : 411  atcattccttggcactggaatatnccccctgcttcaatgccaccaccctcctcgtcctgtc 470
                      |||||||||||||||||||||||||| |||||||||||| |||| |||||||||| ||| ||
Mouse Cyp4a10: 1675 atcattccttggcactggaatattcccctgcttcaaagccagcaccctcctcatcccctc 1734

AD9#3          : 471  tcttctaatctccttccctttttacaaatgcttatgacatcatcaagatccctaaaagctc 530
                      ||||||||| ||||| |||||||||| |||||||||||||||| |||||||||||||||||||
Mouse Cyp4a10: 1735 tcttctaatgtccttacctttttacagatgcttatgacatcattaagatccctaaaagctc 1794

AD9#3          : 531  caagatattttcttcatctaaatatatcttattcaatctaacctactangttccttttga 590
                      ||||||| |||   ||||||||| |||||||||||||||||||||||| ||||||| |||
Mouse Cyp4a10: 1795 caagatagttttcccatctaaatgtatcttattcaatctaacctactaagttccttctga 1854

AD9#3          : 591  attatgaggaattattcattatgtaccatgggctncaaancccaaggcctgaatgtcatt 650
                      |||||||||||| ||||| |||||||||||| || |||||  |||||||||||  |||||
Mouse Cyp4a10: 1855 attatgaggaatcattcaatatgtaccatggtctccaaaatccaaggcctgaacatcatt 1914

AD9#3          : 651  gtgaagccattattcagcctagtatcatnntcacatgacaatnanntaatgtgtccatct 710
                      |||||| |||||||||| ||||||||  |||||||||| | |  ||||||||  |||||
Mouse Cyp4a10: 1915 gtgaagacattattcagcttagtatcattttcacatgacagttacctaatgtgttcatct 1974

AD9#3          : 711  anatctaccctgcatagtc 729
                      | ||||||||||||||||||
Mouse Cyp4a10: 1975 aaatctaccctgcatagtc 1993
```

B 47.3: Summary of sequence alignment of cDNA subclone **AD9#3** with **mouse Cyp4a10**

FluoroDD gel AD (AP2 + ARP18)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AD9	+	++	+	+	710	AD9#3	765	M13 reverse	*Mus musculus* cytochrome P450, family 4, subfamily a, polypeptide 10 (Cyp4a10) (2085 bp)	e-153 398/439 (90%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 48.1: DNA sequence of cDNA subclone AF1#8 (AP10 & ARP13) using M13 forward (-20) primer

```
  1 ACTNNCTATA GGGCNAATTG GGCCCTCTAG ATGCTGCTCG AGNCGGCCGC CAGTGTGATG
 61 GATATCTGCA GAATTCGCCC TTAGCGGATA ACAATTTCAC ACAGGAGTTG CACCATTTAT   ARP13
121 TGGCTATGCT GGAGGAAAGA CCCAGACAGA GAAGAAAAAA CTCAGAGATG TCTTTAAGAA
181 AGGCGACATC TACTTCAACA GCGGAGACCT CCTGATGATC GACCGTGAGA ACTTCGTCTA
241 CTTTCACGAC AGGGTTGGAG ATACTTTCCG GTGGAAAGGA GAGAACGTAG CTACCACAGA
301 AGTCGCTGAC ATCGTGGGAC TGGTAGATTT TGTTGAAGAA GTGAATGTGT ATGGCGTGCC
361 TGTGCCAGGT CATGAGGGTC GAATTGGGAT GGCCTCCCTC AAGATCAAAG AAAACTACGA
421 GTTCAATGGA AAGAAACTCT TTCAACACAT CGCGGAGTAC CTGCCCCAGT TACGCGAGGC
481 CTCGGTTCCT GAGGATACAA GATACCATTG AGATCACTGG GACTTTTAAA CACCGCAAAG
541 TGACCCTGAT GGAAGAGGGC TTCAATCCCA CAGTCATCAA AGATACCTTG TATTTCATGG
601 ATGATGCAGA GAAAACATTT GTGCCATGAC TGAGAACNNT TATAATGCCT AATTGATAAA
661 AATCTGANGC TNTGAATATT CCCTGGTGGN TTAAGCTNAT GACATNNCCA AAAAGAANCT
721 CNATANACCT CCCANANCAC TTNNCTCGTN NAATCAATTT NNNTGGATNG AGGNTTTANG
781 TGNNTTTATT TNNNGAATAT TNTTNAG
```

B 48.2: Sequencing alignment of cDNA subclone **AF1#8** with **mouse very-long-chain acyl-coA synthetase (VLACS)** by BLAST searching against the National Center for Biotechnology Information database

```
AF1#8       : 111  caccatttattggctatgctggaggaaagacccagacagagaagaaaaaactcagagatg 170
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse VLACS: 1310 caccatttattggctatgctggaggaaagacccagacagagaagaaaaaactcagagatg 1369

AF1#8       : 171  tctttaagaaaggcgacatctacttcaacagcggagacctcctgatgatcgaccgtgaga 230
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse VLACS: 1370 tctttaagaaaggcgacatctacttcaacagcggagacctcctgatgatcgaccgtgaga 1429

AF1#8       : 231  acttcgtctactttcacgacagggttggagatactttccggtggaaaggagagaacgtag 290
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse VLACS: 1430 acttcgtctactttcacgacagggttggagatactttccggtggaaaggagagaacgtag 1489

AF1#8       : 291  ctaccacagaagtcgctgacatcgtgggactggtagattttgttgaagaagtgaatgtgt 350
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse VLACS: 1490 ctaccacagaagtcgctgacatcgtgggactggtagattttgttgaagaagtgaatgtgt 1549

AF1#8       : 351  atggcgtgcctgtgccaggtcatgagggtcgaattgggatggcctccctcaagatcaaag 410
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse VLACS: 1550 atggcgtgcctgtgccaggtcatgagggtcgaattgggatggcctccctcaagatcaaag 1609

AF1#8       : 411  aaaactacgagttcaatggaaagaaactctttcaacacatcgcggagtacctgccccagt 470
                   |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Mouse VLACS: 1610 aaaactacgagttcaatggaaagaaactctttcaacacatcgcggagtacctg-cccagt 1668

AF1#8       : 471  tacgcgaggcctcggttcctgaggatacaagataccattgagatcactgggacttttaaa 530
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse VLACS: 1669 tacgcgaggcctcggttcctgaggatacaagataccattgagatcactgggacttttaaa 1728

AF1#8       : 531  caccgcaaagtgaccctgatggaagagggcttcaatcccacagtcatcaaagataccttg 590
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse VLACS: 1729 caccgcaaagtgaccctgatggaagagggcttcaatcccacagtcatcaaagataccttg 1788

AF1#8       : 591  tatttcatggatgatgcagagaaaacatttgtg-ccatgactgagaacnnttataatgcc 649
                   |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse VLACS: 1789 tatttcatggatgatgcagagaaaacatttgtgcccatgactgagaacatttataatgcc 1848

AF1#8       : 650  -taattgataaaaatctgangctntgaatattccctggtggnttaagctnatgacatnnc 708
                    |||||||||||||| ||||| ||| ||||||||||||||||||| || |||| ||||||||  |
Mouse VLACS: 1849 ataattgataaaactctgaagctctgaatattccctggtggttt-agctcatgacatttc 1907

AF1#8       : 709  caaaaagaanctcnatanacctc 731
                   || ||||||| ||| ||| |||||
Mouse VLACS: 1908 cagaaagaaactcgatagacctc 1930
```

B 48.3: Summary of sequence alignment of cDNA subclone **AF1#8** with **mouse VLACS**

FluoroDD gel AF (AP10 + ARP13)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AF1	+	++	+	+	945	AF1#8	990	M13 forward (-20)	*Mus musculus* very-long-chain acyl-coA synthetase (VLACS) (2209 bp)	0 606/623 (97%))

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 49.1: DNA sequence of cDNA subclone AF1#8 (AP10 & ARP13) using M13 reverse primer

```
  1 AGCNTATTTA GGTGACACTA TAGAATACTC AGNCTATGCT NAAGNCTTGG TACCGAGNCN
 61 CGATCCACTA GTAACGGCCG CCAGTGTGCT GGAATNCGCC CTTGTAATAC GACTCACTAT   AP10
121 AGGGCTTTTT TTTTTTTAGT GTTAATATAG TTTATTATGT CTTTAAAAAA ATAAGGCCCT
181 CTCTCCAAGA AGCTTAGTTT GCAAGGACAA ATGGCAGGTG CACATTGAAA AATAATTGTT
241 TCTAAATCTT TTTACTTGNA AAGGNTCAGG TGTACTCCAA AAAAAAAACA AACAAACTAT
301 CCTTTTAATG AATAATTTCC TAAAAATAAA ATCGCACCTT ATAGCCCTCA NNCAAGTTAA
361 AGTTGNNTTC NACGTATGAA GTGGCTCTGC GAGGTCTATC GAGTTTCTTT CTGGANATGT
421 CATGAACTAA ACCCCCCAGG NANNATTCAG ACCTTCAGAN TTTTATCCAN TNATGGCATN
481 ATAAATGTCC TCAGTCATGG NCACAAANGT NTCNTCTGCA TCANCATGAN ATCCAAGTAT
541 CTTGATAACT GGGGGATGAN CCCCCTTCAT CAGGGTCCCT TGCGGTGTTA AAAGTCCAGT
601 GATCCAATGG ATTTNNNTCC CAAGACCGAG GCNCCCCTAC NGNNNGGTCC CCCGAGTGTG
661 AANANTNNTT CCATGACCTT ATTTCTGATT GANGAGCATC ATTNAACTAT ACNGCCAGCA
721 CCATANATNN TTTAAATANT TCCGAGTNNA TTTGACCCTC TNCTTCCG
```

B 49.2: Sequencing alignment of cDNA subclone **AF1#8** with **mouse very-long-chain acyl-coA synthetase (VLACS)** by BLAST searching against the National Center for Biotechnology Information database

```
AF1#8       : 294  aaactatccttttaatgaataatttcctaaaaataaaatcgcaccttatagccctcannc 353
                   ||||||||||||||||||||||||||||||||||||||||||||||||||| | |  ||  |
Mouse VLACS: 2027  aaactatccttttaatgaataatttcctaaaaataaaatcgcaccttatggtctccaatc 1968

AF1#8       : 354  aagttaaagttgnnttcnacgtatgaagtggctctgcgaggtctatcgagtttctttctg 413
                   |||||||||| |  ||| |||| |||||||| ||||||||||||||||||||||||||||
Mouse VLACS: 1967  aagttaaagtgggtttctacgtttgaagtggttctgcgaggtctatcgagtttctttctg 1908

AF1#8       : 414  ganatgtcatgaactaaaccccccaggnannattcagaccttcaganttttatccantna 473
                   || |||||||||| ||||| || |||||  |  ||||||| ||||||| |||||| || | |
Mouse VLACS: 1907  gaaatgtcatgagctaaa-ccaccagggaatattcagagcttcagagttttat-caatta 1850

AF1#8       : 474  tggcatnataaatgtcctcagtcatggncacaaangtntcntctgcatca 523
                   |||||| |||||||| |||||||||||| |||||| || |  |||||||||
Mouse VLACS: 1849  tggcattataaatgttctcagtcatgggcacaaatgttttctctgcatca 1800
```

B 49.3: Summary of sequence alignment of cDNA subclone **AF1#8** with **mouse VLACS**

FluoroDD gel AF (AP10 + ARP13)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AF1	+	++	+	+	945	AF1#8	990	M13 reverse	*Mus musculus* mRNA for very-long-chain acyl-coA synthetase (VLACS) (2209 bp)	1e-56 119/230 (86%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 50.1: DNA sequence of cDNA subclone AF21#5 (AP10 & ARP13) using M13 reverse primer

```
  1 AAGACTATTT AGGTGACCTA TAGAATACTC AAGCTATGCA TCAAGCTTGG TACCGAGCTC
 61 GGATCCACTA GTAACGGCCG CCAGTGTGCT GGAATTCGCC CTTAGCGGAT AACAATTTCA   ARP13
121 CACAGGAGTT GCACCATCAG GCTTGGGGTC TGCAGTGTAC CCTAGGTAAC CAAGCTGACT
181 CCATCCTAAC AGATCTGTAC ACTGCCTTGT TCCTCTGGCC TGTACATCTT CCTGAAGAAT
241 GCTACCTGTC TTCCCTCCAC TCCTGCCTTC CACATACCCT GCAGAACCAC AGCCTTNNCC
301 CCTGCATNCC TGATNCCCAC CGTAATCGCT GCTTCATCTA GGTTCTTACT GACGCCTACC
361 CCTAAGATNC TGCATACCAA CGGGCCACTG TCCCTAGCTT TACTACAAGA TAACTTTCCC
421 CTAAAAAAAA AAAAGCCCTA TAGTGAGTCG TATTACAAGG GCCAATTCTG CAGATATCCA
481 TCACACTGGN CGGCCGCTCC AGCATGCATC NNGAGGGCCC AAATTCGCCC CTATAGTGAG
541 NTCGNNTTAC CAATTCACTG GNCCGTCGGT TTTTANAACC GTCGGTGACT GGGGAAAAAC
601 CCNTGGGCGT TAACCCAANC CTAAATCGGC CTTGGCAGCA AAATTCCCCC TTTTGCCAAG
661 CGGGCGTAAT AGCGANGAGN NCCCACCGAT GCCCTTNCAA CAGTTGCNCN GCCTNNTTGC
721 GNATGGACCC CCCCNGTANG CGCCCCATAA GCGCCNGNCG GNNGGTGGTG GNTACCGCNC
781 NGCGGTGACC GCCTACNNTT NGGCAGCGNC CCCTAGCGNC CCNNNNNTCC TTNNGCTTTT
841 TTTCCTTTCT TTTTNNCCAN NNNNN
```

B 50.2: Sequencing alignment of cDNA subclone **AF21#5** with **mouse cell death-inducing DNA fragmentation factor, alpha subunit-like effector B (Cideb)** by BLAST searching against the National Center for Biotechnology Information database

```
AF21#5      : 131  gcaccatcaggcttggggtctgcagtgtaccctaggtaaccaagctgactccatcctaac 190
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cideb: 850   gcaccatcaggcttggggtctgcagtgtaccctaggtaaccaagctgactccatcctaac 909

AF21#5      : 191  agatctgtacactgccttgttcctctggcctgtacatcttcctgaagaatgctacctgtc 250
                   ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Cideb: 910   agatctgcacactgccttgttcctctggcctgtacatcttcctgaagaatgctacctgtc 969

AF21#5      : 251  ttccctccactcctgccttccacataccctgcagaaccacagccttnnccccctgcatncc 310
                   |||||||||||||||||||||||||||||||||||||||||||||  |||||||| ||
Mouse Cideb: 970   ttccctccactcctgccttccacataccctgcagaaccacagccttgtccccctgcat-cc 1028

AF21#5      : 311  tgatncccaccgtaatcgctgcttcatctaggttcttactgacgcctacccctaagatnc 370
                   |||| ||||||||||||||||||||||||||||||||||||||||||||||||||| |
Mouse Cideb: 1029  tgat-cccaccgtaatcgctgcttcatctaggttcttactgacgcctaccccctaagatcc 1087

AF21#5      : 371  tgcataccaacgggccactgtccctagctttactacaagataactttcccct 422
                   |||||||||||| ||||||||||||||||||||||||||||||||||||||
Mouse Cideb: 1088  tgcataccaac-ggccactgtccctagctttactacaagataactttcccct 1138
```

B 50.3: Summary of sequence alignment of cDNA subclone **AF21#5** with **mouse Cideb**

FluoroDD gel AF (AP10 + ARP13)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AF21	+	++	+	+	340	AF21#5	370	M13 reverse	*Mus musculus* cell death-inducing DNA fragmentation factor, alpha subunit-like effector B (Cideb) (1182 bp)	e-140 285/292 (97%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 51.1: DNA sequence of cDNA subclone AF25#6 (AP10 & ARP13) using M13 forward (-20) primer

```
  1 NNTNNCTCAT ATGGGCNAAT TGGGCCCTAC TAGATGCATG CTCGAGACGG CCGCCAGTGT
 61 GATGGATATC TGCAGAATTC GCCCTTAGCG GATAACAATT TCACACAGGA GTTGCACCAT  ARP13
121 CATCCCTTCC TATCCATACA GCATCCCCAG TATAAATTCT GTGATCTGCA TNCCATCCTG
181 TCTCACTGAG AAGTCCAATT CCCAGTCTAT CCACATGTTA CCNTAGGATA CCTCATCAAG
241 AATCAAAGAC TTCTTTAAAT TTCTCTTTGN ATATACCCAT NACAATTTTT CATGAATTTC
301 TTCCTCTTCC TGTTCAATAA ATGATTACCC TTGCACTAAA AAAAAAAGCC CTTATAGTGA
361 GTCGTATTAC AAGGGGCGAA TTCCAGCCAA CCTGGGCGGG CCGTTNACTT AGGTGGGATC
421 CGAAGCCTTC GGGTAACCCC AGCCNTTTGG NTGGCAATNN ACCTTTGGGA AGGTANTTTC
481 CNTANTNAAG GTGGGTTCAN CCCCCTAAAN AATNAGNNCC NTTGTGGNCN NGTTATNAAT
541 TCAAATTGGG NTTCAATTAA GGCCCTTGGT TTTCCCNTNN GGTTNGGTTG NNAAAANTNT
601 GGNTNNNNNT TCCCGCCTCC CACACNCAAA NTTTNTCCCA ACACANACCA AACAANATTN
661 ACCGNNANGN GCCNNCGGNN NAAGANNCAA NTAAAAAAG
```

B 51.2: Sequencing alignment of cDNA subclone **AF25#6** with **mouse major urinary protein 2 (MUP II)** by BLAST searching against the National Center for Biotechnology Information database

```
AF25#6    : 115 caccatcatcccttcctatccatacagcatccccagtataaattctgtgatctgcatncc 174
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Mouse MUP: 652 caccatcatcccttcctatccatacagcatccccagtataaattctgtgatctgcattcc 711

AF25#6    : 175 atcctgtctcactgagaagtccaattcccagtctatccacatgttaccntaggatacctc 234
              |||||||||||||||||||||||| |||||||||||||||||||| ||||||||||||
Mouse MUP: 712 atcctgtctcactgagaagtccaatt-ccagtctatccacatgttacc-taggatacctc 769

AF25#6    : 235 atcaagaatcaaagacttctttaaatttctctttgnatatacccatnacaatttttcatg 294
              |||||||||||||||||||||||||||||||||||| |||||||||| ||||||||||||
Mouse MUP: 770 atcaagaatcaaagacttctttaaatttctctttg-atatacccatgacaatttttcatg 828

AF25#6    : 295 aatttcttcctcttcctgttcaataaatgattacccttgcact 337
              |||||||||||||||||||||||||||||||||||||||||||
Mouse MUP: 829 aatttcttcctcttcctgttcaataaatgattacccttgcact 871
```

B 51.3: Summary of sequence alignment of cDNA subclone **AF25#6** with **mouse MUP II**

FluoroDD gel AF (AP10 + ARP13)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AF25	++	+	++	++	275	AF25#6	310	M13 forward (-20)	*Mus musculus* major urinary protein 2 (MUP II) (891 bp)	e-103 218/223 (97%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 52.1: DNA sequence of cDNA subclone AF25#7 (AP10 & ARP13) using M13 reverse primer

```
  1 AGAATACTCA AGCTATGCAT CAAGCTTGGT ACCGAGCTCG GATCCACTAG TAACGGCCGC
 61 CAGTGTGCTG GAATTCGCCC TTAGCGGATA ACAATTTCAC ACAGGAGTTG CACCATCATC    ARP13
121 CCTTCCTATC CATACAGCAT CCCCAGTATA AATTCTGTGA TCTGCATTCC ATCCTGTCTC
181 ACTGAGAAGT CCAATTCCAG TCTATCCACA TGTTACCTAG GATACCTCAT CAAGAATCAA
241 AGACTTCTTT AAATTTCTCT TTGATACACC CTTGACAATT TTTCATGAAT TTCTTCCTCT
301 TCCTGTTCAA TAAATGATTA CCCTTGCACC TAAAAAAAAA AAGCCCTATA GTGAGTCGTA
361 TTACAAGGGC GAATTCTGCA GATATCCATC ACACTGGCGG CCGCTCGAGC ATGCATCTAG
421 AGGGCCCAAT TCGCCCTATA GTGAGTCGTA TNACAATTCA CTGGCCGTCG TTTTACAACG
481 TCGTGACTGG GAAAAACCCT GGCCGTNACC CAACTTAATC GCCTTGCAGC CACATCCCCC
541 TTTNGCCAGC TGGCGTAATA GCGAAGAGGC CCGCACCGAT CGCCCTTCCN NACAGTTGCC
601 GCAGCCTGAA TGGCGAATGG ACGCNNCCCT GTAGCGGGNG CATTAAGNGC GGGGGGGTGT
661 GGTGGTNACC NNCAGNGTGA NNGNTANNTT GCCAGNNCNC TAACGCCGGT CTTTTNNTTT
721 TTTTCCTNNT TTTTGCCAGT TGNNGNTNCC NNTNACTTTA NNNGGGGNNN NN
```

B 52.2: Sequencing alignment of cDNA subclone **AF25#7** with **mouse major urinary protein 2 (MUP II)** by BLAST searching against the National Center for Biotechnology Information database

```
AF25#7    : 111 caccatcatcccttcctatccatacagcatccccagtataaattctgtgatctgcattcc 170
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse MUP: 652 caccatcatcccttcctatccatacagcatccccagtataaattctgtgatctgcattcc 711

AF25#7    : 171 atcctgtctcactgagaagtccaattccagtctatccacatgttacctaggatacctcat 230
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse MUP: 712 atcctgtctcactgagaagtccaattccagtctatccacatgttacctaggatacctcat 771

AF25#7    : 231 caagaatcaaagacttctttaaatttctctttgatacaccttgacaattttttcatgaat 290
              |||||||||||||||||||||||||||||||||||| |||| ||||||||||||||||||
Mouse MUP: 772 caagaatcaaagacttctttaaatttctctttgatatacccatgacaattttttcatgaat 831

AF25#7    : 291 ttcttcctcttcctgttcaataaatgattacccttgcac 329
              |||||||||||||||||||||||||||||||||||||||
Mouse MUP: 832 ttcttcctcttcctgttcaataaatgattacccttgcac 870
```

B 52.3: Summary of sequence alignment of cDNA subclone **AF25#7** with **mouse MUP II**

FluoroDD gel AF (AP10 + ARP13)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AF25	++	+	++	++	275	AF25#7	310	M13 reverse	*Mus musculus* major urinary protein 2 (MUP II) (891 bp)	e-114 217/219 (99%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 53.1: DNA sequence of cDNA subclone AF30#4 (AP10 & ARP13) using M13 forward (-20) primer

```
  1 GACTCACTAT AGGGCGAATC GGGCCCTCTA NATGCATGCT CGAGCGGCCG CCAGTGTGAT
 61 GGATATCTGC AGAATCGCCC TTGTATAGAC TCACTATAGG GCTTTTTTTT TTAGTATCGG   AP10*
121 GGGTGATCAT AGAGGTCCTC TTCTAATNNT GTGTGCTTAA TTATNATGAT GAAGNTNGGA
181 GTAATCAATC CTCGATGGTC CGGGACATCG GTCGATGTAT GAGGTCGATG ATGTTNGGAG
241 TNATGTNCGG AAGGNAGGGA TNNGGGGTAC CCGGCCAATA TATTAGTTNG TGCCTACCTT
301 NNNAANNGGT GCCAACNTCC CTGGTGGTGN AAANTTGGTT AATCCCGCTT AAAGGGGCNN
361 TANTNCCCAG CCACANCTGG GCNNGCCCGT TAAACTAGTT GGGATCCGAG CCTNNGGTAC
421 CAAGCCCTTG AATGGCATTN NCTTGAAGTT ATNNTTATNA NNTGTTCACC NNAAANTTAG
481 NCTTGGGCGT TTAATTNNTN GGGTNNNTTA ANNNGGTTTT CNNGGTTGGT TTGAAAATTT
541 GGTTTATAAA AAAAAAAAAA AAANNCCCAA AAAAAAAAAA ANNAAAAAAG CCCNGGGAAA
601 CCCTTTTTNA GTTTGTTTAA ANNNNTTGGG GGGGGTGGCC CTTAANTTTG AAANTGGAAC
661 CCTAACTTTT CAACTTTTTA ATTNGCGTTN GGGCCCCNTT NNNTGNNCCN NATTTTTCTC
721 AATTTTGGGG NNAAANNCTN GNTTNCNTNC CACAACNNNG CCTTTTAANT TTAANNTNTG
781 GGCCCAANNC CCCCCNGGGG GGAAAANGGC NGNNTTNGGC GNTNTTNNNN NNNNNNNNNN
841 NNNNNNNNNN NNNNNN
```

B 53.2: Sequencing alignment of cDNA subclone **AF30#4** with **mouse mRNA for suppressor of actin mutations (SAC1 gene)** by BLAST searching against the National Center for Biotechnology Information database

```
AF30#4      : 1  gactcactatagggcgaatcgggccctctanatgcatgctcgagcggccgccagtgtgat 60
               ||||||||||||||||||| |||||||||| ||||||||||||||||||||||||||||
Mouse SAC1: 4  gactcactatagggcgaattgggccctctagatgcatgctcgagcggccgccagtgtgat 63

AF30#4      : 61 ggatatctgcagaa-tcgcccttgt 84
               ||||||||||||||| ||||||||||
Mouse SAC1: 64 ggatatctgcagaattcgcccttgt 88
```

B 53.3: Summary of sequence alignment of cDNA subclone **AF30#4** with **mouse SAC1**

FluoroDD gel AF (AP10 + ARP13)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AF30	+	++	+	+	240	AF30#4	290	M13 forward (-20)	*Mus musculus* mRNA for suppressor of actin mutations (SAC1 gene) (2029 bp)	1e-29 82/85 (96%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 54.1: DNA sequence of cDNA subclone AF30#5 (AP10 & ARP13) using M13 reverse primer

```
  1 ANNTNNTNTT ANNGTGANAN NCATAGAAAT ANCTACAAGN NCTNATGCAT CAAGCNTTGN
 61 GNTACAGCAN CANCNGGCAT NCACAATNAN NTNACAAGGC CNGNCAANNT GTGCATGGCA
121 ANNAGCCCAT TNNCAGGCAT ANCCAATTTC AACAACAAGG NAGTNGCNAN CCAATCNATT   ARP13*
181 CNAAGTAGNN CAACTATATA TTGCCGCTAC CCCAATCCCT CCTTCCAACA TAACTCCAGC
241 ATCATCAACC TCATACATCA ACCAATCTCC CAAACCATCA AGATTAATTA CTCCAACTTC
301 ATCATAATAA TTAAGCACAC AAATTAAAAA AACCTCTATA ATCACCCCCA ATACTAAAAA    AP10
361 AAAAAAGCCC TATAGTGAGT CGTATTACAA GGGCCAATTC TGCAGATATC CATCANACTG
421 GCGGCCGCTC NAGAATGCAT TTAAGAGGNN CCCAATNCCG CCCCNNNNNG TGGANGTTNG
481 NTATNNACAA ATTTTCAANN TNGGGCCCGG TTTGGTNTTT TTAACANAAA CCGTNTTNGG
541 TNGGAAACNT TGGGGGNAAA AAAAAACCCC CCTNGGGNAC CGTTTTAAAC CCCCCAAAAN
601 NCCTTTTAAA ATTTTNCGGC CCCNTTTGGG NNANGGACAA AAANANTTNT TCCCCCCCCA
661 NTTTTNTTTA GGGCNCNCAA GGCCNNTTGG GGNNCNGNNT ATAANTTNAA NNNCCNGGNA
721 NNNANAANNN GGNGCCCCCC CGGGCAAAAA CCNCNGGNNN ATTTTCGGGG CCCACCCCTT
781 GTNNNNNNNN AAAAACANAG GNNTTTTTGG GGNNNNNNNN NNNNNNNNTN GGNNAANNNT
841 NGNNGGGGGG NNNNNANNNN NTTNNGNGGG NNNNNNNNNN NNNCCNNNNN NNNNCCTTNT
901 NTTGTNNNTN TTTAA
```

B 54.2: Sequencing alignment of cDNA subclone **AF30#5** with **mouse mitochondrion** by BLAST searching against the National Center for Biotechnology Information database

```
AF30#5                : 183   aagtagnncaactatatattgccgctaccccaatccctccttccaacataactccagcat 242
                              ||||||  ||||||||||||||||||||||||||||||||||||||||||||||||| |||
Mouse mitochondrion: 13624 aagtagcacaactatatattgccgctaccccaatccctccttccaacataactccaacat 13683

AF30#5                : 243   catcaacctcatacatcaaccaatctcccaaaccatcaagattaattactccaacttcat 302
                              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 13684 catcaacctcatacatcaaccaatctcccaaaccatcaagattaattactccaacttcat 13743

AF30#5                : 303   cataataattaagcacacaaattnnnnnnnncctctataatcacccccaatact 355
                              ||||||||||||||||||||||        ||||||||||||||||||||||
Mouse mitochondrion: 13744 cataataattaagcacacaaattaaaaaaacctctataatcacccccaatact 13796
```

B 54.3: Summary of sequence alignment of cDNA subclone **AF30#5** with **mouse mitochondrion**

FluoroDD gel AF (AP10 + ARP13)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AF30	+	++	+	+	240	AF30#5	290	M13 reverse	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	1e-72 170/173 (98%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 55.1: DNA sequence of cDNA subclone AH1#6 (AP11 & ARP19) using M13 forward (-20) primer

```
  1 CCTCACTATA GGGCGAATNG GGCCCTCTAG ATGCATGCTC GAGCGGCCGC CAGTGTGATG
 61 GATNTCTGCA GAATTCGCCC TTAGCGGATA ACAATTTCAC ACAGGATTTT GGCTCCCCTG    ARP19
121 CAGAAATCCC AAGCCCTGAT GTGTACTGCC TGCTCTTTAG CTCTCCTGAT TTAGTGATTT
181 ATGAGGACCT CGGGAGCATG ACACTTGCTG TATGACTCCT ACATCCATAG ATCCTTAAAA
241 TCACATCTCA TGATGCTGAT AGAAGATTTT AAGAAAGATA TTAATAACCC CCTTGAAAAA
301 ATACAGGAGA ACAGATTTAA AAGGGAGAAG GTTTTAAAGA GAAAACACAA AAATCCATTA
361 AAAAATTACA AGAAAACACA ACCAAACAGG TGAAGGAATT GAACAAAACT ATCCAGGATC
421 TAAAAATGGA AGTAGAAACA ATACAGGAAT CACAATGGGA GAAAACTTCA GAGATATAAA
481 TCCTAGGAAA GAGATCAGAA GTCATAGATT CAGGCATCAC CAAAAGAATA CAAGAAATAG
541 AAGAGAGACT TTCATGTACA GAAGATACCA TAGAAAACAT TGACACAACA GTCAAAGAAA
601 GTGCAAAATG CAAAAACTTT CTAACTCAAA ACATCCAAGC AATCCAGAAC GCAATGAGAA
661 GAACAACCCT AAGGATAACA NGNATAGANA AGANCAAAAA TNCCNAACTN AANNGGCCAG
721 TAAATATTNN CAACAAAANT AGAAAANANA ACATTCNGAN NNTAAANGAT AAAAAAAAAG
781 CCTANNGTGA GTNNNNTACA GGGCNAATNC ANCCANNGGC GCCGTTACTN TNNATCNANC
841 TNGGNNC
```

B 55.2: Sequencing alignment of cDNA subclone **AH1#6** with **mouse EST** by BLAST searching against the National Center for Biotechnology Information database

```
AH1#6     : 237 aaaatcacatctcatgatgctgatagaagattttaagaaagatattaataacccccttga 296
              ||||||||  |||||||||| |||||||  ||  |||||  ||  ||  ||  |||||  |  |||||  |
Mouse EST:  16  aaaatcacttctcatgatgatgatagaggaatttaaaaaggacataaataaactccttta  75

AH1#6     : 297 aa-aaatacaggagaacagatttaaa-agggagaaggttttaaagagaaaacacaaaaat 354
              ||  ||||||||||||||||  |  ||||  ||  |||||    ||||||||  ||||||||||||
Mouse EST:  76  aagaaatacaggagaacacaggtaaacagctagaagcccttaaagaggaaacacaaaaat 135

AH1#6     : 355 ccattaaaaaattacaagaaaacacaaccaaacaggtgaaggaattgaacaaaactatcc 414
              ||  |||||  |  |||||  ||||||||||  ||||||||||||||||  ||||||||||  ||||
Mouse EST: 136 cccttaaagagttacaggaaaacacaatcaaacaggtgaaggaaatgaacaaaaccatcc 195

AH1#6     : 415 aggatctaaaaatggaagtagaaacaatacaggaatcacaatgggagaaaacttcagaga 474
              |  ||||||||||||||||  ||||||||||  ||  ||||||||  ||||||  |||    |||
Mouse EST: 196 aagatctaaaaatggaactagaaacaataaagaaatcacaaagggagacaaccctggagt 255

AH1#6     : 475 tataaatcctaggaaagagatcagaagtcatagattcaggcatcaccaaaagaatacaag 534
              ||  |||  ||||||||||||||||||  ||||||||||  ||  ||||||||||  ||||||||||
Mouse EST: 256 tagaaaacctaggaaagagatcaggagtcatagatgcaagcatcaccaacagaatacaag 315

AH1#6     : 535 aaatagaagagagactttca---tgtacagaagataccatagaaaacattgacacaacag 591
              |  ||||||||||||  |  |||    ||  |  ||||||||||||||||||||||||||||||
Mouse EST: 316 acatagaagagagaatctcagagggtgcggaagataccatagaaaacattgacacaacag 375

AH1#6     : 592 tcaaagaaagtgcaaaatgcaaaaactttctaactcaaaacatccaagcaatccagaacg 651
              |||||||||  |||||||  ||||||  |  |||||  |||||||||    |  |||||||  ||
Mouse EST: 376 tcaaagaaaatgcaaaaaccaaaaagctcctaacccaaaacatctgggaaatccaggaca 435

AH1#6     : 652 caatgagaagaacaaccctaaggataa 678
              ||||||||||||  |||  ||||||||||||
Mouse EST: 436 caatgagaagaccaaacctaaggataa 462
```

B 55.3: Summary of sequence alignment of cDNA subclone **AH1#6** with **mouse EST**

FluoroDD gel AH (AP11 + ARP19)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AH1	+	-	+	+	720	AH1#6	750	M13 forward (-20)	vm09c03.r1 Knowles Solter mouse blastocyst B1 *Mus musculus* cDNA clone IMAGE:989668 5' similar to gb:U15647_cds1 *Mus musculus* (624 bp)	2e-82 377/447 (84%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 56.1: DNA sequence of cDNA subclone AI1#5 (AP6 & ARP4) using M13 forward (-20) primer

```
  1 GGGCCCTCTA GATGCATGCT CGAGCGGCCG CCAGTGTGAT GGATATCTGC AGAATTCGCC
 61 CTTAGCGGAT AACAATTTCA CACAGGAGCT AGCAGACCCA GGGAATGATC AAGGAACTCA   ARP4
121 TCTCAGAACT GGATGAGAGG ACATTGATGG TGCTGGTGAA TTACATCTAC TTTAAAGGCA
181 AATGGAAGAT ATCCTTTGAC CCCCAGGACA CATTTGAGTC TGAGTTCTAC TTGGATGAGA
241 AGAGATCTGT GAAGGTNCCC ATGATGAAAA TGAAGTTACT GACCACNACG CCACTTCCGT
301 GATGAGGAGC TATCGGTGCT CTGTGTTGGA GCCGAAGTAC ACAGGAANAT GCCAGCGNNC
361 TGCTNCATCC TCCCTGACCA GGGCAGGATG CANNNCAGGT GGAAGCCAGC TTACAACCAG
421 NAGACCCTGA GGAAATGGAN GGAAAACTNT TGTCNTCCCA NCCAAATATG AGGATNCTAA
481 ACCTGCCCCT AGTNCNNCAN CGCCTATTAA CTTCAGGNTT GGANGACGAT TTCCTNNNAN
541 ANGTGTGGAT TAAGGAANNT NNCANNGATA CATTCTGACC CATCTGTGGA TCTCATGAAA
601 CCGGGGATNC TTGTGNTGNT GTCTCAANGT GGTCCCAATC GTGNCTATTN CATTGCATGT
661 GGTAATGNAC ACNNNTCTCN CNANCCTAGT TTGGTTGCTA CNNAGGGTAA TTGNATNNNC
721 NTTTCATAAG GGGCNCTANT ACCNANGCA
```

B 56.2: Sequencing alignment of cDNA subclone **AI1#5** with **mouse serine (or cysteine) proteinase inhibitor (SPI)** by BLAST searching against the National Center for Biotechnology Information database

```
AI1#5     : 93  cagacccagggaatgatcaaggaactcatctcagaactggatgagaggacattgatggtg 152
Mouse SPI: 610 cagacccagggaatgatcaaggaactcatctcagaactggatgagaggacattgatggtg 669

AI1#5     : 153 ctggtgaattacatctactttaaaggcaaatggaagatatcctttgacccccaggacaca 212
Mouse SPI: 670 ctggtgaattacatctactttaaaggcaaatggaagatatcctttgacccccaggacaca 729

AI1#5     : 213 tttgagtctgagttctacttggatgagaagagatctgtgaaggtncccatgatgaaaatg 272
Mouse SPI: 730 tttgagtctgagttctacttggatgagaagagatctgtgaaggttcccatgatgaaaatg 789

AI1#5     : 273 aagttactgaccacnacgccacttccgtgatgaggagctatcggtgctctgtgttggagc 332
Mouse SPI: 790 aagttactgaccac-acgccacttccgtgatgaggagctatc-gtgctctgtgttggagc 847

AI1#5     : 333 cgaagtacacaggaanatgccagcgnnctgctncatcctccctgaccagggcaggatgca 392
Mouse SPI: 848 tgaagtacacaggaa-atgccagcgccctgct-catcctccctgaccagggcaggatgca 905

AI1#5     : 393 nnncaggtggaagccagcttacaaccagnagaccctgaggaaatgganggaaaact 448
Mouse SPI: 906 --gcaggtggaagccagcttacaaccag-agaccctgaggaaatgga-ggaaaact 957
```

B 56.3: Summary of sequence alignment of cDNA subclone **AI1#5** with **mouse SPI**

FluoroDD gel AI (AP6 + ARP4)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AI1	+	-	+	+	1100	AI1#5	1110	M13 forward (-20)	*Mus musculus* serine (or cysteine) proteinase inhibitor clade A, member 3K (Spi2) (1645 bp)	0 343/356 (96%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 57.1: DNA sequence of cDNA subclone AI1#5 (AP6 & ARP4) using M13 reverse primer

```
  1 GGNCNTATTT AGGTGACACT ATAGAATACT CAAGCTATGC ATCAAGCTTG GTACCGAGCT
 61 CGGATCCACT AGTAACGGCC GCCAGTGTGC TGGAATTCGC CCTTGTAATA CGACTCACTA   AP6
121 TAGGGCTTTT TTTTTTTTCC CGGTATTGGA ACTCTGTGTA TTTGCAAATG CCTAGGTTTG
181 GGGTACAGAC ACATAAAGAG GAGTCACCCG GGAAGAAGAA TAAAGGCAGA TTCCAGGTNC
241 AGAAATATGC ACAGATGCTC CCAAGACAAC CGAGAGCCTG ACACTGAGAG TCCAAAGTCT
301 TATGTGCATG TGGGATCACA GAGATAGTTA ANTGTACCTG TCTAAGNCCA ACTTTGCAAC
361 AGCCAANTCA GAGCTTGAAT GACAGGATGT ATATNNGAGA TCCACATGCA NNACTTTGTN
421 NCCACAGCCC TGNNACAGNA ACCCATGAGN AACTTGGNTG AGCTTTTNNG TNNTACTTGG
481 GNGTNATTCG ACTTTGGCCC NTANAANGAG NGATACTCTT TATCCATCTT NGNNGTGTAT
541 AATNANTAAT TCANNAACAT TGTAAANTGG GCCCCCNGTT AGAAAAATTN GNNATCAGNC
601 NTATNNGGTN AANNTTNGTT CCCCTTACCC AAATTGNCNT TTCCCTTTNT TCCCCCNTTT
661 GGTTANGNTC ATTCTGCCAT NNNNNTNGNN TTGNTNNATN NNTTTNNTNT TTNNNNNCTC
721 NNNNNNTTTT TTNNNANNCA TCCTTNATTA TCNANNCGNN CANCTTTNTT NTCGGNGNTT
781 TCTNCGGGNN GGANTCCCNN NAAATNNNTT NNNTNNNTAN GNANTNNNNA ATNNTNN
```

B 57.2: Sequencing alignment of cDNA subclone **AI1#5** with **mouse serine (or cysteine) proteinase inhibitor (SPI)** by BLAST searching against the National Center for Biotechnology Information database

```
AI1#5     : 142  ggtattggaactctgtgtatttgcaaatgcctaggtttggggtacagacacataaagagg 201
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1629 ggtattggaactctgtgtatttgcaaatgcctaggtttggggtacagacacataaagagg 1570

AI1#5     : 202  agtcacccgggaagaagaataaaggcagattccaggtncagaaatatgcacagatgctcc 261
                 ||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Mouse SPI: 1569 agtcacccgggaagaagaataaaggcagattccaggttcagaaatatgcacagatgctcc 1510

AI1#5     : 262  caagacaaccgagagcctgacactgagagtccaaagtcttatgtgcatgtgggatcacag 321
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse SPI: 1509 caagacaaccgagagcctgacactgagagtccaaagtcttatgtgcatgtgggatcacag 1450

AI1#5     : 322  agatagttaantgtacctgtctaagnccaactttgcaacagccaantcagagcttgaatg 381
                 |||||||||| |||| ||||||||| |||||||||||||||||| ||||||||||||||
Mouse SPI: 1449 agatagttaa-tgtagctgtctaag-ccaactttgcaacagccaa-tcagagcttgaatg 1393

AI1#5     : 382  acaggatgtatatnngagatccacatgca 410
                 |||||||||||| ||||||||||||||
Mouse SPI: 1392 acaggatgtatata-gagatccacatgca 1365
```

B 57.3: Summary of sequence alignment of cDNA subclone **AI1#5** with **mouse SPI**

FluoroDD gel AI (AP6 + ARP4)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AI1	+	-	+	+	1100	AI1#5	1110	M13 reverse	*Mus musculus* serine (or cysteine) proteinase inhibitor clade A, member 3K (Spi2) (1645 bp)	e-123 262/269 (97%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 58.1: DNA sequence of cDNA subclone AI18#6 (AP6 & ARP4) using M13 forward (-20) primer

```
  1 CCNTCCACTA TNNGGCGAAT NNCGGGCCCT ACTAGATGCT GCTCAGACGG CCGCNNGNGT
 61 GAATGGATTT CTGNNGAANT TCGNCCCTTG NTAAATACGA CCTCACTATA GGGCTTTTTT  AP6
121 TTTTTTCCCT GTTTCTTCAG GGACTTCACC TTTATTGGGG ACGACCGCTT TGGGTGGGAC
181 GGGATCTAGG GCTCCTGGGC TTGCCAAGTA GGGCCCGCAC CTGCCTGATC TGCCATTCGA
241 CACTGGATTT CGCTGTGCCA CCCAAGGCAC TGTACTGTTC CACGCTGTGA CTGTAGTCCC
301 ACACGTGACT CACATCACCT GAGAACAGGG GACTGATGGT CTGCTAGCTC CTGTGTGAAA
361 TTGTATCCGC TAAGGGCGAA TTCCAGACAC ACTGGCGGCC GTTACTAGTG GATCCCGAGC
421 TCCGGTACCC AAGCTTGATG CATAAGCTTG AGTATCCCTA TAGTGTCACC TAAAATAGCT
481 GGGCGTAATC ATGGTCATAG CTGTNNCCTG TGTGAAATTG TTATCCGCTC ACAATNCCAC
541 ACAACATACG AGCCGGAAGC ATAAAGTGTA AAGCCTGGGG NGCCTAATGA GTGACCTAAA
601 CTTNAATTAA TNGCGTTGCG CTCCCTGCCC GCTTCCCAGT NNGGGAAACC TGTTGTGCCA
661 GCTGCATTAN TGAATTGGGC ANNGCGCGGG GAGAGGCGGT TNGGNTNTGG GNCNNNTTTC
721 GNTTTNTNNT NNTGANNTGC TGNGCTNGNT NTTNGGTGNG NNANGGTNTT NNTNNTTNNN
781 GGTAATNNGT NTNNNNAATN NNNATNNNCC GAAAATTTNA CCAGGGNNCA AGGCAAACTT
841 ANNGNGNNTN TT
```

B 58.2: Sequencing alignment of cDNA subclone **AI18#6** with **mouse argininosuccinate lyase (Asl)** by BLAST searching against the National Center for Biotechnology Information database

```
AI18#6    : 167  gctttgggtgggacgggatctagggctcctgggcttgccaagtagggcccgcacctgcct 226
               ||||||||||| |||||||||||||||||||||||||  |||||||||||||||||||
Mouse Asl: 1494 gctttgggtggg-cgggatctagggctcctgggcttgc--agtagggcccgcacctgcct 1438

AI18#6    : 227  gatctgccattcgacactggatttcgctgtgccacccaaggcactgtactgttccacgct 286
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Asl: 1437 gatctgccattcgacactggatttcgctgtgccacccaaggcactgtactgttccacgct 1378

AI18#6    : 287  gtgactgtagtcccacacgtgactcacatcacctgagaacaggggactgatggtctgcta 346
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Mouse Asl: 1377 gtgactgtagtcccacacgtgactcacatcacctgagaacaggggactgatggtctgc-a 1319

AI18#6    : 347  gctcctgt 354
               ||||||||
Mouse Asl: 1318 gctcctgt 1311
```

B 58.3: Summary of sequence alignment of cDNA subclone **AI18#6** with **mouse Asl**

FluoroDD gel AI (AP6 + ARP4)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AI18	++	+	++	++	270	AI18#6	300	M13 forward (-20)	*Mus musculus* argininosuccinate lyase (Asl) (1589 bp)	6e-84 184/188 (97%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 59.1: DNA sequence of cDNA subclone AI18#6 (AP6 & ARP4) using M13 reverse primer

```
  1 NCNTATTTAG GTGACACTAT AGAATACTCA AGCTATGCAT CAAGCTTGGT ACCGAGCTCG
 61 GATCCACTAG TAACGGCCGC CAGTGTGCTG GAATTCGCCC TTAGCGGATA ACAATTTCAC    ARP4
121 ACAGGAGCTA GCAGACCATC AGTCCCCTGT TCTCAGGTGA TGTGAGTCAC GTGTGGGACT
181 ACAGTCACAG CGTGGAACAG TACAGTGCCT TGGGTGGCAC AGCGAAATCC AGTGTCGAAT
241 GGCAGATCAG GCAGGTGCGG GCCCTACTGC AAGCCCAGGA GCCCTAGATC CCGCCCACCC
301 AAAGCTGCTC CCCAATAAAG TGAAGCCCTG AAGAAACAGG AAAAAAAAAA AAGCCCTATA    AP6
361 GTGAGTCGTA TCACAAGGGC GAATNNCTGC AGATATCCAT CACAACTGGC GGCCGCTCGA
421 GCATGCCATC TAGAGGGCCC CAATTNCCGC CCTATAGTGA GTCGTATTAC AATTCACTGG
481 NCCGTCGTTT TACAACGTGG TGACTGGNGA AAACCCTGGC GTTACCCAAC TTAATTGCCT
541 TGCAGCACAT CCCCCTTNCG CCAGCTGGCG TAATAACGAA GAGGCNNGAA CCGATCGCCC
601 TTNCCAACAG TNGGCCCNGC TGAATGGCGA ATGGACCCCC CCTGTAGNNC CCCATTAAGC
661 GCNGGNGGTG GTGGTGGTAA CCCCCNNNNT GAACGTTNNA CTTNGCCAAG GNNNNTANNC
721 NNNNNTTTCT TTTTTTTTTT TCCCCNTTCT TTTTNNNCAA NNNTTGGCGG GCTTTCACAC
781 TTNNNCCNNT TNNNTTGGGG GCTCCNTTTN NGNNNNNTNT NNNNNNNNAN NNNCTNACTN
841 NNNNNNNTGN NN
```

B 59.2: Sequencing alignment of cDNA subclone **AI18#6** with **mouse argininosuccinate lyase (Asl)** by BLAST searching against the National Center for Biotechnology Information database

```
AI18#6    : 121  acaggagctagcagaccatcagtcccctgttctcaggtgatgtgagtcacgtgtgggact 180
               |||||||||  ||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Asl: 1311 acaggagct-gcagaccatcagtcccctgttctcaggtgatgtgagtcacgtgtgggact 1369

AI18#6    : 181  acagtcacagcgtggaacagtacagtgccttgggtggcacagcgaaatccagtgtcgaat 240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Asl: 1370 acagtcacagcgtggaacagtacagtgccttgggtggcacagcgaaatccagtgtcgaat 1429

AI18#6    : 241  ggcagatcaggcaggtgcgggccctactgcaagcccaggagccctagatcccgcccaccc 300
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Asl: 1430 ggcagatcaggcaggtgcgggccctactgcaagcccaggagccctagatcccgcccaccc 1489

AI18#6    : 301  aaagctgctccccaataaagtgaagccctgaagaaac 337
               |||||||||||||||||||| ||||||||||||||||
Mouse Asl: 1490 aaagctgctccccaataaagtggagccctgaagaaac 1526
```

B 59.3: Summary of sequence alignment of cDNA subclone **AI18#6** with **mouse Asl**

FluoroDD gel AI (AP6 + ARP4)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AI18	++	+	++	++	270	AI18#6	300	M13 reverse	*Mus musculus* argininosuccinate lyase (Asl) (1589 bp)	e-110 215/217 (99%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 60.1: DNA sequence of cDNA subclone AJ1#4 (AP6 & ARP14) using M13 forward (-20) primer

```
  1 CNTCACTATA GGGCGAATTG GGCCCTCTAG ATGCNTGCTC GAGCGGCCGC CAGTGTGATG
 61 GATATCTGCA GAATTCGCCC TTGTAATACG ACTCACTATA GGGCTTTTTT TTTTTTCCTC   AP6
121 TGAAGAGCTT TATTAATGTG CTGGACTGTA CCATACAGCA AGGGTNCAAG TCACTTTAGG
181 AAGAAACAGC AGTATGTTCA ATGATCAAGA AGTAGAAACC AGATTGGTAA CATNTAGTGG
241 TCCACCATAC TGGTCTTNNA GGGGCTGACG ATTTAAGAAA CATGGGCTCA CAGCAGCATG
301 GTCCAAGCTA AAGTCTCTGA ACAGAAACCA AAAGANGTGG TAGTTGCCCT GCTTGGGATC
361 CTACTANNCT TGGGNNNGCC CCAGTCAAGG ANGGTNCAAG TTANGTCTGG CCCTCANAGC
421 TCCTCTGGGA ACTTNCCTGC TTCTCTGCTC TCCGTGCCAT TCTGANNNTT CTTGGCAGTN
481 TCTCTGTCCA NACTGTACCC GACCCTCTTT NNCTCACCCC ATCCGTGTNN TAAACATCTC
541 CAGTATGTNC TACTGTNNAT NGGCCAAGAG CACCCTGCCT GGGATNCCGG CTGCACTGCC
601 CTTGGGCATA TGTTAGCCAC TGCTNCTCCC TGTNNCCGGN ANGCCAGAGG ACCTCTCANT
661 GAGAAGACCC AAAACANNCT TNCNGAGATG TCACNTCCCA GCGCTGACT
```

B 60.2: Sequencing alignment of cDNA subclone **AJ1#4** with **mouse carboxylesterase** by BLAST searching against the National Center for Biotechnology Information database

```
AJ1#4                          : 117  cctctgaagagctttattaatgtgctggactgtaccatacagcaagggtncaagtcactt 176
                                      ||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Mouse carboxylesterase: 2005 cctctgaagagctttattaatgtgctggactgtaccatacagcaagggttcaagtcactt 1946

AJ1#4                          : 177  taggaagaaacagcagtatgttcaatgatcaagaagtagaaaccagattggtaacatnta 236
                                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Mouse carboxylesterase: 1945 taggaagaaacagcagtatgttcaatgatcaagaagtagaaaccagattggtaacattta 1886

AJ1#4                          : 237  gtggtccaccatactggtcttnnaggggctgacgatttaagaaacatgggctcacagcag 296
                                      |||||||||||||||||||  |||||||| ||||||||||||||||||||||||||||
Mouse carboxylesterase: 1885 gtggtccaccatactggtcttgtaggggctgatgatttaagaaacatgggctcacagcag 1826

AJ1#4                          : 297  catggtccaagctaaagtctctgaacagaaaccaaaagangtggtagttgccctgcttgg 356
                                      |||||||| |||||||||||||||||||||| |||||| || ||||||||||||||| |
Mouse carboxylesterase: 1825 catggtccaggctaaagtctctgaacagaaacaaaaagaagttgtagttgccctgctt-g 1767

AJ1#4                          : 357  gatcctactanncttgggnnngccccagtcaaggganggtncaagttangtctggccctca 416
                                      ||| |||||| |||||| | ||||||| |||| ||| | || || |||||| ||||
Mouse carboxylesterase: 1766 gatgctactattcttgggttgg-cccagtc-aggaaggtcc-aggtaggtctgg-gctca 1711

AJ1#4                          : 417  nagctcctctgggaacttnncctgcttctctgctctccgtgccattct--gannnttcttg 474
                                      |||||||||| ||||||| |||||||||||||||||| ||||||||||   |   ||||||
Mouse carboxylesterase: 1710 gagctcctct-ggaactttcctgcttctctgctctcggtgccattcttgaatttttcttg 1652

AJ1#4                          : 475  gcagtntctctgtcca-nactgtacccgaccct 506
                                      ||||| |||||||||| |||||| ||||||||
Mouse carboxylesterase: 1651 gcagtgtctctgtccagaactgtagccgaccct 1619
```

B 60.3: Summary of sequence alignment of cDNA subclone **AJ1#4** with **mouse carboxylesterase**

FluoroDD gel AJ (AP6 + ARP14)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AJ1	++	+	++	++	1200	AJ1#4	990+220	M13 forward (-20)	Mouse carboxylesterase (Carboxylesterase) (2006 bp)	e-123 354/393 (90%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 61.1: DNA sequence of cDNA subclone AJ1#5 (AP6 & ARP14) using M13 reverse primer

```
  1 GNNCATTTAG GTGANCCTAT AGAATACTCA AGACTATGCA TCAAGCTTGG TACCGAGCTC
 61 GGATCCACTA GTAACGGCCG CCAGTGTGCT GGAATTCGCC CTTAGCGGAT AACAATTTCA   ARP14
121 CACAGGATCC ATGACTCCTT CTTCCCACAA AGGCCCCAGA AGCTCCTAGN CAAACAAGNC
181 AATTCCCCAC TGTGCCCTAC CTCTTGGGAG TCACCAACCA TGAGTTTGGC TGGCCTTCTA
241 NCTCAAATTC TGGAATATCC TGGATAAGAT GGAACCATTT GAGACCAGGA AGACCTGTTG
301 GAGAATTCAA GGCCCTTATT AGACCNCATA TGCCACTGCC CCCTGAGATC ATGCCCNNCC
361 GTCATAGATG ANNACCTAGA CAATGGCTCA NGATGAATCA GCTACCAGGT ATGCCCTCCA
421 GGAATTGCTG GGTGATATCA CATNGGTCAT TCCTACCCTN GNNCTTCTCA AAATACCCTN
481 AAAGATGCTG GGNTGCCCCT GTTTTCTTGG TAACGANGTN NCANGCATAC ANCCCANGTT
541 NCTTTTTGNC AAAGGTTNCA AGCCCAAGCC TGGGGNTGAA AGGNCTGAAC CATTCCCTCN
601 TGAGNAATGN CCTTTGTTTT TTGGGAANNT TCNTTTTCCT NNCTGATTGA GANNTTTCCC
661 CTCCTGGCCC TTCCCNAGAN GGCCAACNGA AGNNAAAAAA AANGCANNNT NGAGCCCTGA
721 CCCNTTGANT GGGNCNCAAT TGGGNNACCN NNTNTNTTGG ACNNCCCCCN NGGGAAAAAA
781 TTNACAACAA AANAGNNNAA AAANNNGGGG GNGNGTGGGG CCNTTTNCNC TTTGNGGG
```

B 61.2: Sequencing alignment of cDNA subclone **AJ1#5** with **mouse carboxylesterase** by BLAST searching against the National Center for Biotechnology Information database

```
AJ1#5                    : 131  atgactccttcttcccacaaaggccccagaagctcctagncaaacaagncaattccccac 190
                                |||||||||||||||||||||||||||||||||||||||||||| |||||||| ||||||||||
Mouse carboxylesterase: 947  atgactccttcttcccacaaaggccccagaagctcctag-caaacaag-caattccccac 1004

AJ1#5                    : 191  tgtgccctacctcttgggagtcaccaaccatgagtttggctggccttctanctcaaattc 250
                                |||||||||||||||||||||||||||||||||||||||||||| ||||| |||||||||
Mouse carboxylesterase: 1005 tgtgccctacctcttgggagtcaccaaccatgagtttggctggc-ttcta-ctcaaattc 1062

AJ1#5                    : 251  tggaatatcctggataagatggaaccatttgagaccaggaagacctgttggagaattcaa 310
                                |||||||||||||||||||||||||| ||||||| ||||||||||||||||||||||||||
Mouse carboxylesterase: 1063 tggaatatcctggataagatggaac-atttgag-ccaggaagacctgttggagaattcaa 1120

AJ1#5                    : 311  ggcccttattagaccncatatgccactgccccctgagatcatgcccnnccgtcatagatg 370
                                |||||||||||| | ||||||| |||||||||||||||||||||| |||||||||||||
Mouse carboxylesterase: 1121 ggcccttattag--cccatatgcaactgccccctgagatcatgccca-ccgtcatagatg 1177

AJ1#5                    : 371  annacctagacaatggctcangatgaatcagctaccaggtatgccctccaggaattgctg 430
                                | |||||||||||||||| |||||||||||||| |||||||||||||||||||||||||
Mouse carboxylesterase: 1178 aatacctagacaatggctca-gatgaatcagctacaaggtatgccctccaggaattgctg 1236

AJ1#5                    : 431  ggtgatatcacatnggtcattcctaccctngnnncttctcaaaatacccctnaaagatgctg 490
                                |||||||||||||| |||||||||||| ||| | |||||||||||| ||| |||||||||
Mouse carboxylesterase: 1237 ggtgatatcacattggtcattccta-ccttgatcttctcaaaata-ccttcaagatgctg 1294

AJ1#5                    : 491  ggntgcccctgttttcttg 509
                                || || ||||||||||||||
Mouse carboxylesterase: 1295 gg-tg-ccctgttttcttg 1311
```

B 61.3: Summary of sequence alignment of cDNA subclone **AJ1#5** with **mouse carboxylesterase**

FluoroDD gel AJ (AP6 + ARP14)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AJ1	++	+	++	++	1200	AJ1#5	990+220	M13 reverse	Mouse carboxylesterase (Carboxylesterase) (2006 bp)	e-127 353/379 (93%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 62.1: DNA sequence of cDNA subclone AJ2#10 (AP6 & ARP14) using M13 forward (-20) primer

```
  1 NNATAGGGCG AATNGGGCCT CTAGATGCAT GCTCGAGACG GCCGCCAGTG TGATGGATAT
 61 CTGCAGAATN NCGCCCTTAG CGGATAACAT TTCACACAGG ATCCATGACT CCACTTCCTG   ARP14
121 ACACAGCTAA GTTGCTTGTC TTTACCTCCA GGCTTTCGGC CGTTGCCTGG ACTTCAATCA
181 TGGTGGCTGA CCTTCCCTTT CTTGCTTTGC TTCTCCTCAA AGAGATAATA GAGACAATGA
241 CCAGTCTTTC CTCATAGATC AAGTATGGGG AGAGCCCTCA GCTATGGTAT TCCTGTATTT
301 TGGTGACTTA TTTAAGTAAA TTTCCTGGGA CAATCCAGAT TTGAAAGATT CTGTCTTCTT
361 GTNGTCATAA ACTATTAAAA TGCTTGGTGG TCACCAAAGT ATTTGACATA AAAATAAATA
421 AATAAATCAT TCAGGCCACC TTTTACACCA GAAATCACAG GAAAGCCCTG GGCCCCAGCC
481 ATCTGCTGAG TGTTAGTNGA GAAGATGGAT CCTAAGCCAG CTGAAGACTG AGTGCAGGCT
541 GTGGGGAGGT TCTTGCTGAG TAGCTGGCTT TGTGGTAAGC TGCTAGCAGC CTTACAGNGG
601 TGGCGAAGCA GCCCCCCCTT NGGATGCAGA GCAGCCNTCT ACAATNATTN NTGACCNTNA
661 AAGGTAGANN ATNGNACNTT TTTGTNGGTA TGTGTGTGNT NTGCNTCTCT NCTATTNNAG
721 NTGANTTTTT TTTTNNTNGA GAACNNNGNC CCCAGAGNTN GGCCNTGNAN CTNNTGANTN
781 CNTCAGNNNT NCCANANGNN NNNNGGTTAA NNGNTTNGNN CTGANNNNNN NGNNTAGNTG
841 GNACCTTTNG NTTTAANGAC AAGNTAAGAA ATTTGTGANC NTTT
```

B 62.2: Sequencing alignment of cDNA subclone **AJ2#10** with **peroxisomal acyl-coA oxidase (AOX)** by BLAST searching against the National Center for Biotechnology Information database

```
Mouse AOX: 111  ccacttcctgacacagctaagttgcttgtctttacctccaggctttcggccgttgcctgg 170
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AJ2#10   : 2732 ccacttcctgacacagctaagttgcttgtctttacctccaggctttcggccgttgcctgg 2791

Mouse AOX: 171  acttcaatcatggtggctgaccttccctttcttgctttgcttctcctcaaagagataata 230
                |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
AJ2#10   : 2792 acttcaatcatggtggctgaccttccctttcttgccttgcttctcctcaaagagataata 2851

Mouse AOX: 231  gagacaatgaccagtctttcctcataga-tcaagtatggggagagccctcagctatggta 289
                |||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
AJ2#10   : 2852 gagacaatgaccagtctttcctcatagactcaagtatggggagagccctcagctatggta 2911

Mouse AOX: 290  ttcct--gtatttt-ggtgacttatttaagtaaatttcctgggacaatccagatttgaaa 346
                ||||   ||| ||| ||||||||  |||||||||||| ||||||||||||||||||||
AJ2#10   : 2912 ttccgtagtaatttaggtgactttgttaagtaaatt-cctgggacaatccagatttgaa- 2969

Mouse AOX: 347  gattct-gtcttcttgtngtcataaactattaaaatgcttggtggtcaccaaagtatttg 405
                || | | |||||  |||| ||||||||||||||||||||||||||| |  | ||||||||||||
AJ2#10   : 2970 gactatcgtcttgttgttgtcataaactattaaaatgcttggtcgctaacaaagtatttg 3029

Mouse AOX: 406  acataaaaataaataaataaatcattcaggccacctttacaccagaaatcacaggaaag 465
                |||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
AJ2#10   : 3030 acataaaaataaataaataaatcattcaggccacctttacaccaggaatcacaggaaag 3089

Mouse AOX: 466  ccctgggccccagccatctgctgagtgttagtngagaagatggatcctaagccagctgaa 525
                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AJ2#10   : 3090 ccctgggccccagccatctgctgagtgttagttgagaagatggatcctaagccagctgaa 3149

Mouse AOX: 526  gactgagtgcaggctgt-ggggaggttcttgct-gagtagctggctttg-tggtaagctg 582
                || |||||||||||||| |||||||||||||| |||||||||||||| ||||||||||
AJ2#10   : 3150 gaatgagtgcaggctgtgggggaggttcttgctggagtagctggctttgttggtaagctg 3209

Mouse AOX: 583  ctagcagccttacagnggtggcgaagcag 611
                ||||||||||||||| ||||||||||||
AJ2#10   : 3210 -tagcagccttacag-ggtggcgaagcag 3236
```

B 62.3: Summary of sequence alignment of cDNA subclone **AJ2#10** with **mouse AOX**

FluoroDD gel AJ (AP6 + ARP14)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AJ2	+	++	+	+	1100	AJ2#10	1130	M13 forward (-20)	*Mus musculus* peroxisomal acyl-coA oxidase (AOX) (3778 bp)	0 481/509 (94%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 63.1: DNA sequence of cDNA subclone AJ2#10 (AP6 & ARP14) using M13 reverse primer

```
  1 AATAGAATAC TCAAGCTATG CATCAAGCTT GGTACCGAGC TCGGATCCAC TAGTAACGGC
 61 CGCCAGTGTG CTGGANTTCG CCCTTGTAAT ACGACTCACT ATAGGGCTTT TTTTTTTTTC      AP6
121 CAGTCATTTA AATTTATTGG CTATTTTCCA GAATATGAAT TACTGTCAAA TAGAAACCCC
181 ACAGGTGACA TGCTGCTTAC TGTCAATCTT CAGGGATTAT CAGTCATTCC AGGAGAAAGG
241 TTAAGACTGT GTCAGCAAAT CTGATGGCTT TGACTTGATG TCATTTCCAC TCCATTAATA
301 CTGTGTTCTC AGAACTGCTC TGAAAGAACT GTTCTCACGA TGCCAATGCC ACAGACACAG
361 ACTTAATAGG TCACAATGAT CTCTCTCACT GTGCTTTTAA GAACAAAGAG TTCCAACTAG
421 CCAGGCATGT CAGCGCAAAC CTGTAACCCC AGCATCTGGG AGGCTGAGGA TCAGANGTCA
481 AGGCCACTCT GGGCCCTGTC TCAAGAAAAA AAAATCACTA CATAGAAAAA AGCATACACA
541 CACATACCAC AANNNNNTCC ATACTCTANC TTTANNGTCA GAATGATTGT AGAGGCTGCT
601 CTGCATCCNA AGGGGGGCTG CTCCCCCACC CTGNTANNGG CTGCNTAGCC AGCNTNANCC
661 ACAAAAGCCC NAGCCNTACC TCAGNCNNAN GAANNCNNNN CCCCCCACAA GCCCC
```

B 63.2: Sequencing alignment of cDNA subclone **AJ2#10** with **peroxisomal acyl-coA oxidase (AOX)** by BLAST searching against the National Center for Biotechnology Information database

```
Mouse AOX: 122  agtcatttaaatttattggctatttt-ccagaatatgaattactgtcaaatagaaacccc 180
                |||||||||||||||||| |||||||| |||||||||| ||||||||||||||||||||
AJ2#10   : 3744 agtcatttaaatttatttgctatttttccagaatatgacttactgtcaaatagaaacccc 3685

Mouse AOX: 181  acaggtgacatgctgcttactgtcaatcttcagggattatcagtc----attccaggaga 236
                |||||||||||||||||||||||||||||||||||||||||||| |    ||||||||||
AJ2#10   : 3684 acaggtgacatgctgcttactgtcaatcttcagggattatcagaccttgattccaggaga 3625

Mouse AOX: 237  aaggttaagact-gtgtcagcaaatctgatggctttgacttgat--gtcatttccactcc 293
                |||||||||||| |||||||||||||||||||||||||||||   |||| |||||||||
AJ2#10   : 3624 aaggttaagacttgtgtcagcaaatctgatggctttgacttgaatagtcaattccactcc 3565

Mouse AOX: 294  attaatactgtgttctcagaactgctctgaaagaactgttctcacgatgccaatgccaca 353
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AJ2#10   : 3564 attaatactgtgttctcagaactgctctgaaagaactgttctcacgatgccaatgccaca 3505

Mouse AOX: 354  gacacagacttaataggtcacaatgatctctctcactgtgcttttaagaacaaagagttc 413
                |||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
AJ2#10   : 3504 gacacagacttaataggtcacaatgatctctct-actgtgcttttaagaacaaagagttc 3446

Mouse AOX: 414  caactagccaggcatgtcagcgcaaacctgtaaccccagcatctgggaggctgaggatca 473
                | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AJ2#10   : 3445 cgactagccaggcatgtcagcgcaaacctgtaaccccagcatctgggaggctgaggatca 3386

Mouse AOX: 474  gangtcaaggcca-ctctgggccctgtctcaag 505
                || |||||||||| ||||||||||||||||||
AJ2#10   : 3385 gaggtcaaggccaactctgggccctgtctcaag 3353
```

B 63.3: Summary of sequence alignment of cDNA subclone **AJ2#10** with **mouse AOX**

FluoroDD gel AJ (AP6 + ARP14)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AJ2	+	++	+	+	1100	AJ2#10	1130	M13 reverse	*Mus musculus* peroxisomal acyl-coA oxidase (AOX) (3778 bp)	e-170 376/393 (95%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 64.1: DNA sequence of cDNA subclone AJ9#1 (AP6 & ARP14) using M13 forward (-20) primer

```
  1 CCCACNNTAA TNNAGANNGG AGTANNNGGC GCCTCTANAN NNNCNTNNGC TNCNGCANNC
 61 GGNCNGCCCA GTGTGATGGA TATNCGNNNN NAATTNCACC CTGATAATAA CACCTNNACT   AP6
121 ATAGGGCTTT TTTTTTTTC CCTACTTAAA AAGGAATTAA GTTTATTCTT TCACAGTTAT
181 ACCCACAGAT ACAGTTATGA GCTCATACAT TACAGGGAAT TTTGTTCCAT TTTCACAGGG
241 TACACATCCA GTAAAACCAG CTTCCAGATC AGGAACTAGC CTTACGAACA TCTCTGCCGT
301 TACCCCACAG CATCCTTTTG CTTTGCTGAT ACCCAGCACT TCTTCACACC AGAGTAGTTA
361 GGTTGCATTA GTCTTTGACT GTTGCTGACC TTTCTACAAA TGGAGTCATG GATCCTGTGT
421 GAAATTGNNA TCCGCTAAGG GCGAATTCCA GCACACTGGC GGCCGTTACT AGTGGATCCG
481 AGCTCGGTAC CAAGCTTGAT GCATAGCTTG AGTATTCTAT AGTGTCACCT AAATAGCTTG
541 GCGTAATCAT GGTCATAGCT GTTTACTGTG TGAAATTGTT ATCCGCTCAC CATTCCACAC
601 AATATTACGA GCCGGGAAGC ATAAAGTGTA AAGCCTGGGG GTGCCTAATG AGTGAGGCTA
661 ANTCACATTT AATTGCGTTT GCGCCTCAAN TGGCCCCGCT TNCNAGTTNG GGNANACCTT
721 GTNNNGTGCC ANNCTNNNNA TNNTNTGANN TNNNGGGNAA CCGCCNCCGG GGNANANGCN
781 NGNTTNNNNN NTANTNNGGG CGCTTTTTCC GCTTTTTTTG CATTNNNTGN ANNTTCCTTG
841 NNNCTNNGNT TTTTTNGGNN TGNNGNNNAN CNNNTNTTTG GTTTTNNTTN NNNNGNNNNT
901 NTTGNNNAT
```

B 64.2: Sequencing alignment of cDNA subclone **AJ9#1** with **mouse catalase** by BLAST searching against the National Center for Biotechnology Information database

```
AJ9#1          : 444 aattccagcacactggcggccgttactagtggatccgagctcggtaccaagcttgatgca 503
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse catalase: 31  aattccagcacactggcggccgttactagtggatccgagctcggtaccaagcttgatgca 90

AJ9#1          : 504 tagcttgagtattctatagtgtcacctaaatagcttggcgtaatcatggtcatagctgtt 563
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse catalase: 91  tagcttgagtattctatagtgtcacctaaatagcttggcgtaatcatggtcatagctgtt 150

AJ9#1          : 564 tactgtgtgaaattgttatccgctcaccattccacacaatattacgagccgggaagcata 623
                     | |||||||||||||||||||||||| |||||||||| | |||||||| |||||||||
Mouse catalase: 151 tcctgtgtgaaattgttatccgctcacaattccacacaaca-tacgagcc-ggaagcata 208

AJ9#1          : 624 aagtgtaaagcctgggggtgcctaatgagtga 655
                     ||||||||||||| ||||||||||||||||||
Mouse catalase: 209 aagtgtaaagcct-ggggtgcctaatgagtga 239
```

B 64.3: Summary of sequence alignment of cDNA subclone **AJ9#1** with **mouse catalase**

FluoroDD gel AJ (AP6 + ARP14)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AJ9	+	-	+	+	320	AJ9#1	360	M13 forward (-20)	if84h05.x1 Kaestner ngn3 - - subtracted *Mus musculus* cDNA 3'similar to SW:CATA_CAMJE Q59296 CATALASE (280 bp)	1e-93 206/212 (97%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 65.1: DNA sequence of cDNA subclone AJ9#1 (AP6 & ARP14) using M13 reverse primer

```
  1 CNCANNTTAN GGTGNNCNCT ATANGAATAC TACAAGNCTA TGCATCAAGC NTTGGTANCN
 61 GNACATCGGC ATNCNATCAN TCACAGGCCG CCAANTGTGC NTGGCAATNC GCCCTTCACG
121 GCANACAATT TCACACAGGA TCCATGACTC CATTTGTAGA AAGGTCAGCA ACAGTCAAAG   ARP14
181 ACTAATGCAA CCTAACTACT CTGGTGTGAA GAAGTGCTGG GTATCAGCAA AGCAAAAGGA
241 TGCTGTGGGG TAACGGCAGA GATGTTCGTA AGGCTAGTTN CCTGATCTGG AAGCTGGTTT
301 TACTGGATGT GTACCCTGTG AAAATGGAAC AAAATTCCCT GTAATGTATG AGCTCATAAC
361 TGTATCTGTG GGTATAACTG TGAAAGAATA AACTTAATTC CTTTTTAAGA GGAAAAAAAA
421 AAAAGCCCTA TAGTGAGTGT ATTACAAGGG CGAATNNTGC AGATATNCAT CACACTGGCG
481 GCCGNTCGAG CATGCTTNTA GAGGGCCCAA TTAGCCCTAT AGTGAGTNTA TNACATTNCT
541 GGCGTCGTTT TNNNGTCTGN TGGGAANCCT GGNGTNNNNT NNTNTTGACC TCCCTNNNNT
601 GGNTNNTCAN AGCGNNTNNT NNTGNCCNNA TGNNNTNGNC NNGTNNNCTN NNNNGGTNTG
661 GTNTNNTNNN NNNNNNNNTT
```

B 65.2: Sequencing alignment of cDNA subclone **AJ9#1** with **mouse suppressor of actin mutations (SAC1 gene)** by BLAST searching against the National Center for Biotechnology Information database

```
AJ9#1     : 444 acaagggcgaatnntgcagatatncatcacactggcggccgntcgagcatgcttntagag 503
              ||||||||||||  |||||||| ||||||||||||||||| |||||||||| | |||||
Mouse SAC: 88  acaagggcgaattctgcagatatccatcacactggcggccgctcgagcatgcatctagag 29

AJ9#1     : 504 ggcccaattagccctatagtgagt 527
              ||||||||| ||||||||||||||
Mouse SAC: 28  ggcccaattcgccctatagtgagt 5
```

B 65.3: Summary of sequence alignment of cDNA subclone **AJ9#1** with **mouse SAC1**

FluoroDD gel AJ (AP6 + ARP14)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AJ9	+	-	+	+	320	AJ9#1	360	M13 reverse	*Mus musculus* suppressor of actin mutations (SAC1 gene) (2029 bp)	2e-24 77/84 (91%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 66.1: DNA sequence of cDNA subclone AL2#8 (AP7 & ARP15) using M13 forward (-20) primer

```
  1 CCTCACTATA GGGCGAATTG GGCCCTCTAG ATGCATGCTC GAGCGGCCGC CAGTGTGATG
 61 GATATCTGCA GAATTCGCCC TTGTAATACG ACTCACTATA GGGCTTTTTT TTTTTCGAGT   AP7*
121 CATTGATTTT CCTTTTATTT AAAGGTCAAG CCATGGTCTC TGCAGCAGAT GGAGACACTG
181 AGCGTGAGAT TTGGTCCATT TATTATTTCT GTCTGTCCAT CTGTCCGCGT GTTTTCCAGT
241 ACGCAGACTG CAATCCCACA GACGCTTTTC CTCCAAGCTC CCTTGGGCTG GTATCTCAGG
301 AAATAGCGCT GATCCACGGA ATGGAACGTT ACAGAGCACA TGACTCCGCT GTGAAATGAC
361 ACTACACTCA GTCCTTCATT CCACCGTGAG AGGTTAAGGT TTCTAATTTT ATATGGGGTA
421 GAAAGTCCTG TGTGAAATNG TTATCCGCTA AGGGCGAATN CCAGCACACT GGCGGCCGTT
481 ACTAGTGGAT CCGAGCTCGG TACCAAGCTT GATGCATAGC TTGAGTATTC TATAGTGTCA
541 CCTAAATAGC TTGGCGTAAT CATGGTCATA GCTGTTTNCT GTGTGANNTN GTNATCCGCT
601 CACAANNCCA CACAACATAC GAGCCNGANG CATAANNTGT AAAGCCTGGG NNGCCTANTG
661 AGTGAGCTAA CTCACATTAA TTGCGTNGCN CNCACTGNCC GCCTTTCAGT NNGNACACCT
721 GTCGTGCAGC TGCATNAATG AATNGCCAAC GCNCGNGGAA GGCGGGTTGC GTTTNGGNNC
781 NNTCCGNNNT CNCTAATGAN NNNGGCCNNG NTNTG
```

B 66.2: Sequencing alignment of cDNA subclone **AL2#8** with **mouse hydroxysteroid (17-beta) dehydrogenase 11 (Hsd17β11)** by BLAST searching against the National Center for Biotechnology Information database

```
AL2#8          : 116  cgagtcattgattttccttttatttaaaggtcaagccatggtctctgcagcagatggaga 175
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Hsd17b11: 1685 cgagtcattgattttccttttatttaaaggtcaagccatggtctctgcagcagatggaga 1626

AL2#8          : 176  cactgagcgtgagatttggtccatttattatttctgtctgtccatctgtccgcgtgtttt 235
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Hsd17b11: 1625 cactgagcgtgagatttggtccatttattatttctgtctgtccatctgtccgcgtgtttt 1566

AL2#8          : 236  ccagtacgcagactgcaatcccacagacgcttttcctccaagctcccttgggctggtatc 295
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Hsd17b11: 1565 ccagtacgcagactgcaatcccacagacgcttttcctccaagctcccttgggctggtatc 1506

AL2#8          : 296  tcaggaaatagcgctgatccacggaatggaacgttacagagcacatgactccgctgtgaa 355
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Hsd17b11: 1505 tcaggaaatagcgctgatccacggaatggaacgttacagagcacatgactccgctgtgaa 1446

AL2#8          : 356  atgacactacactcagtccttcattccaccgtgagaggttaaggtttctaattttatatg 415
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Hsd17b11: 1445 atgacactacactcagtccttcattccaccgtgagaggttaaggtttctaattttatatg 1386

AL2#8          : 416  gggtagaa 423
                      ||||||||
Mouse Hsd17b11: 1385 gggtagaa 1378
```

B 66.3: Summary of sequence alignment of cDNA subclone **AL2#8** with **mouse HSD17β11**

FluoroDD gel AL (AP7 + ARP15)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AL2	+	++	+	+	350	AL2#8	390	M13 forward (-20)	*Mus musculus* hydroxysteroid (17-beta) dehydrogenase 11 (HSD17β11) (1713 bp)	e-171 308/308 (100%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 67.1: DNA sequence of cDNA subclone AL3#3 (AP7& ARP15) using M13 forward (-20) primer

```
  1 ANCNTCACTA TACGGGCAAT CGGGCCCTNC TAGATGCTGC TCGAGACGGC CGCCAGTGTG
 61 ATGGATATCT GCAGAATTCG CCCTTAGCGG ATAACAATTT CACACAGGAC TTTCTACCCC   ARP15
121 ATATAAAATT AGAAACCTTA ACCTCTCACG GTGGAATGAA GGACTGAGTG TAGTGTCATT
181 TCACAGCGGA GTCATGTGCT CTGTAACGTT NCCATTCCGT GGATCAGCGC TATTTCCTGA
241 GATACCAGCC CAAGGGAGCT TGGAGGAAAA GCGTCTGTGG GATTGCAGTC TGCGTACTGG
301 AAAACACGCG GACAGATGGA CAGACAGAAA TAATAAATGG ACCAAATCTC ACGCTCAGTG
361 TCTCCATCTG CTGCAGAGAC CATGGCTTGA CCTTTAAATA AAAGGAAAAT CAATGACTCG
421 AAAAAAAAAA AGCCCTATAG TGAGTCGTAT TACAAGGGCG AATNNCCAGC ACACCTGGCG
481 GCCGTTACTA TTGGATCCGA GCTCGGTACC AAGCTTGATG CATACCCTNN GAGTATNCTA
541 TNNTGTNACC TAAATAGCTT GGCGTAATCN NTGGTCATAG CCTGTTNTNC TGTNGTGAAA
601 ATTNGTCATN NNCNCTCNNC AATTTTACAC NNCNAACNNT ANCNANCNNC GGAAANNCAT
661 AAAANNTGTA NACCNCCNTG GGGGTGCCNT NNTGNAANNN AGNCTANCTC ANNTTTAATT
721 GGCTTTGCCC CTCANTGCCC GCCTTTACAT TTTNGGGANN ACTTGTNCTT NCCATCTGCT
781 TTTAATTAAA TTCGGCNNAA CCCCCCGGGN ANAGGNCGNT NNNTT
```

B 67.2: Sequencing alignment of cDNA subclone **AL3#3** with **mouse hydroxysteroid (17-beta) dehydrogenase 11 (Hsd17β11)** by BLAST searching against the National Center for Biotechnology Information database

```
AL3#3          : 114  ctaccccatataaaattagaaaccttaacctctcacggtggaatgaaggactgagtgtag 173
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Hsd17b11: 1380 ctaccccatataaaattagaaaccttaacctctcacggtggaatgaaggactgagtgtag 1439

AL3#3          : 174  tgtcatttcacagcggagtcatgtgctctgtaacgttnccattccgtggatcagcgctat 233
                      ||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Mouse Hsd17b11: 1440 tgtcatttcacagcggagtcatgtgctctgtaacgtt-ccattccgtggatcagcgctat 1498

AL3#3          : 234  ttcctgagataccagcccaagggagcttggaggaaaagcgtctgtgggattgcagtctgc 293
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Hsd17b11: 1499 ttcctgagataccagcccaagggagcttggaggaaaagcgtctgtgggattgcagtctgc 1558

AL3#3          : 294  gtactggaaaacacgcggacagatggacagacagaaataataaatggaccaaatctcacg 353
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Hsd17b11: 1559 gtactggaaaacacgcggacagatggacagacagaaataataaatggaccaaatctcacg 1618

AL3#3          : 354  ctcagtgtctccatctgctgcagagaccatggcttgacctttaaataaaaggaaaatcaa 413
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Hsd17b11: 1619 ctcagtgtctccatctgctgcagagaccatggcttgacctttaaataaaaggaaaatcaa 1678

AL3#3          : 414  tgactcg 420
                      |||||||
Mouse Hsd17b11: 1679 tgactcg 1685
```

B 67.3: Summary of sequence alignment of cDNA subclone **AL3#3** with **mouse HSD17β11**

FluoroDD gel AL (AP7 + ARP15)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AL3	+	++	+	+	345	AL3#3	380	M13 forward (-20)	*Mus musculus* hydroxysteroid (17-beta) dehydrogenase 11 (HSD17β11) (1713 bp)	e-166 306/307 (99%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 68.1: DNA sequence of cDNA subclone AL3#3 (AP7& ARP15) using M13 reverse primer

```
  1 GNCNTATTTA NGTGACACTA TAGAATACTC NAGACTATGC NTCAAGCTTG GTACCGAGAC
 61 TCGGATCCAC TAGTAACGGC CGCCAGTGTG CTGGAATTCG CCCTTGTAAT ACGACTCACT   AP7
121 ATAGGGCTTT TTTTTTTTTC GAGTCATTGA TTTTCCTTTT ATTTAAAGGT CAAGCCATGG
181 TCTCTGCAGC AGATGGAGAC ACTGAGCGTG AGATTTGGTC CATTTATTAT TTCTGTCTGT
241 NCATCTGTCC GCGTGTTTTC CAGTACGCAG ACTGCAATCC CACAGACGCT TTTCCTCCAA
301 GCTCCCTTGG GCTGGTATCT CAGGAAATAG CGCTGATCCA CGGAATGGAA CGTTACAGAG
361 CACATGACTC CGCTGTGAAA TGACACTACA CTCAGTCCTT CATTCCACCC GTGAGAGGTT
421 AAAGGTTTCT AATNTTTATA TGGGGTANAA AAGTCCTGTG TGAAATTGTT ATCCGCTAAN
481 GGGCCGAATT CTGCAAATAT CNATCACACT NGGNGGNNGN TNNANCNTGN NNTNTTANAG
541 GGNNNTNNNC TATNNTNANT NTANNNTNTG GGCNTCNNNN ATTNTNNTGN AAACCTNNNT
601 NNNTATNNNN NTNNNNTNNN NTNTNNNNNG NNTNNNNNNT NNNNNN
```

B 68.2: Sequencing alignment of cDNA subclone **AL3#3** with **mouse hydroxysteroid (17-beta) dehydrogenase 11 (Hsd17β11)** by BLAST searching against the National Center for Biotechnology Information database

```
AL3#3          : 140  cgagtcattgattttccttttatttaaaggtcaagccatggtctctgcagcagatggaga 199
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Hsd17b11: 1685 cgagtcattgattttccttttatttaaaggtcaagccatggtctctgcagcagatggaga 1626

AL3#3          : 200  cactgagcgtgagatttggtccatttattatttctgtctgtncatctgtccgcgtgtttt 259
                      ||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
Mouse Hsd17b11: 1625 cactgagcgtgagatttggtccatttattatttctgtctgtccatctgtccgcgtgtttt 1566

AL3#3          : 260  ccagtacgcagactgcaatcccacagacgcttttcctccaagctcccttgggctggtatc 319
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Hsd17b11: 1565 ccagtacgcagactgcaatcccacagacgcttttcctccaagctcccttgggctggtatc 1506

AL3#3          : 320  tcaggaaatagcgctgatccacggaatggaacgttacagagcacatgactccgctgtgaa 379
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Hsd17b11: 1505 tcaggaaatagcgctgatccacggaatggaacgttacagagcacatgactccgctgtgaa 1446

AL3#3          : 380  atgacactacactcagtccttcattccacccgtgagaggttaaaggtttctaatntttat 439
                      |||||||||||||||||||||||||||| |||||||||||| |||||||||| |||||
Mouse Hsd17b11: 1445 atgacactacactcagtccttcattccacc-gtgagaggttaa-ggtttctaat-tttat 1389

AL3#3          : 440  atggggta 447
                      ||||||||
Mouse Hsd17b11: 1388 atggggta 1381
```

B 68.3: Summary of sequence alignment of cDNA subclone **AL3#3** with **mouse HSD17β11**

FluoroDD gel AL (AP7 + ARP15)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AL3	+	++	+	+	345	AL3#3	380	M13 reverse	*Mus musculus* hydroxysteroid (17-beta) dehydrogenase 11 (HSD17β11) (1713 bp)	e-155 304/308 (98%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 69.1: DNA sequence of cDNA subclone AO1#2 (AP5 & ARP10) using M13 forward (-20) primer

```
  1 CCNCNCACNT ATANGGCAAT TGGGCCCTCT AGATGCATGC TCGAGACGGC CGCCAGTGTG
 61 ATGGATATCT GCAGAATTCG CCCTTAGCGG ATAACAATTT CACACAGGAG ATCTCAGACT   ARP10
121 ACATGCCAGA CTGTCCTGGT CAACGCCCAA GGGTTACCAC AGAACATTCA AGATCAGGCC
181 AGACACTTGG GGGTGATGGC AGGCGACATC TACTCCGTAT TCCGCAATGC TGCCTCCTTT
241 AAGGAAGTGT CCGATGGCGT CCTCACATCT AGCAAGGGGC AGCTGCAGAA AATGAAGGAA
301 TCCTTAGATG AAGTTATGGA TTACTTTGTT AACAACNACG CCCCTCAACT GGCTGGTAGG
361 TCCCTTTTAT CCTCAGTCTA CCGAGGTGAA CAAGGCCAGC CNTGAAGGTC CCAGCAGTCT
421 GAGGTCNAAN GCTCAGTANA CCCCTCCCTT GTCACCAGAG CATGATGTTG CTGGCCCAGA
481 TGACCCCCTT TTGCTGCATT GAAATNAACT TGGTAGATGG CTTTAGCTTA GAAAAGCAGC
541 TTNTNNAGAN CCCAGGGCCT CATTATGGTC CCTCACCGCT CAGTTATGGT CTTGCCCCAG
601 CTGNNCCTGG CACANGAGTT TCTTACCCTG GCTNGTGAGT GGCCTGTGTN AGTTTNNTGA
661 NGANCTGNAN GAACCTATAC GCTNGATGCN CTTAAGTTTN GTNTGTNGCC TTTGTTTTGT
721 NNTGGGNTGT ACAGTTTGTC TGGANNGANT AAGATNCTTT NNNNGNNAAA AAAAAGNNCT
781 NTNNTNNNTN TTTTTCGGGG GGGANTTCAC ANNNNGNNN
```

B 69.2: Sequencing alignment of cDNA subclone **AO1#2** with **mouse adipose differentiation related protein (ADFP)** by BLAST searching against the National Center for Biotechnology Information database

```
AO1#2      : 115  cagactacatgccagactgtcctggtcaacgcccaagggttaccacagaacattcaagat 174
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse ADFP: 1036 cagactacatgccagactgtcctggtcaacgcccaagggttaccacagaacattcaagat 1095

AO1#2      : 175  caggccagacacttgggggtgatggcaggcgacatctactccgtattccgcaatgctgcc 234
                  ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse ADFP: 1096 caggccaaacacttgggggtgatggcaggcgacatctactccgtattccgcaatgctgcc 1155

AO1#2      : 235  tcctttaaggaagtgtccgatggcgtcctcacatctagcaaggggcagctgcagaaaatg 294
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse ADFP: 1156 tcctttaaggaagtgtccgatggcgtcctcacatctagcaaggggcagctgcagaaaatg 1215

AO1#2      : 295  aaggaatccttagatgaagttatggattactttgttaacaacnacgcccctcaactggct 354
                  |||||||||||||||||||||||||||||||||||||||||| ||||| ||||||||||||
Mouse ADFP: 1216 aaggaatccttagatgaagttatggattactttgttaacaac-acgcctctcaactggct 1274

AO1#2      : 355  ggtaggtccctttatcctcagtctaccgaggtgaacaaggccagccntgaaggtcccag 414
                  |||||||||||||||||||||||||||||||||||||||||||||| ||||||| ||||
Mouse ADFP: 1275 ggtaggtccctttatcctcagtctaccgaggtgaacaaggccagcc-tgaaggt-ccag 1332

AO1#2      : 415  cagtctgaggtcnaangctcagtanacccctcccttgtcaccagagcatgatgttgctgg 474
                  |||||||||||| || |||||||| ||||||| ||||||||||||||||||||||||||||
Mouse ADFP: 1333 cagtctgaggtc-aaagctcagtaaacccct-ccttgtcaccagagcatgatgttgctgg 1390

AO1#2      : 475  cccagatgaccccctttgctgcattgaaatnaacttggtagatggctttagcttagaaa 534
                    |||||||| |||||||||||| |||||||| ||||||||||||||||||||||||||||
Mouse ADFP: 1391 -ccagatga-ccccttttgctgtattgaaattaacttggtagatggctttagcttagaaa 1448

AO1#2      : 535  agcagcttntnnaganccccagggcctcattatggtccctcaccgctcagttatggtcttg 594
                  |||||||| |  ||| || |||||||||||||||||| ||||| |||||||||||||||||
Mouse ADFP: 1449 agcagcttct-tagaaccaagggcctcattatggtcactcacagctcagttatggtcttg 1507

AO1#2      : 595  ccccagctgnncctggcacangagtt-tcttaccctggctngtgagtggcctgtgtnagt 653
                  |||||||||  |||||||| ||||| ||||| |||||||| |||||||||||||| |||
Mouse ADFP: 1508 ccccagctggccctggcacaggagttctctta-cctggctggtgagtggcctgtgttagt 1566

AO1#2      : 654  ttnntganganctgnangaaccta 677
                  |  ||| || ||| | |||||||
Mouse ADFP: 1567 cttgtgaggacctggaggaaccta 1590
```

B 69.3: Summary of sequence alignment of cDNA subclone **AO1#2** with **mouse ADFP**

FluoroDD gel AO (AP5 + ARP10)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AO1	+	++	+	+	700	AO1#2	720	M13 forward (-20)	Mouse adipose differentiation related protein (ADFP) (1680 bp)	0 530/564 (93%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 70.1: DNA sequence of cDNA subclone AO1#5 (AP5 & ARP10) using M13 reverse primer

```
  1 ACCCNNNTAG TAGNATACAC ATATAANATC ATCAGACATG TNACATCAGA CTGNGNNANN
 61 CCAGACTNGA ATNACCNAGN NNAACGGNCC GCNNGTGTGC TGGAAATNCN CCCTTAGACG
121 GANTAACAAT TTCACACAAG GAGATCTCAG ACAANAANNG ANNNATAGAA AACCTTGGTA   ARP10
181 ACNAAGAGTA GGATGGTGAT GTGAACNTGG AAGGAATGTT GACCTTAAAG GAAGACCTGT
241 TAATGTAGGG TTAGGAGAGT GATGACTCTA CTAGAGTTAT TACTAACNCT TAAAGTCTNN
301 CTGTGTTNNA AACCANCAAT GNCAAAGTCA GATATAGTGA ATAGAAACTA TGNCAATGTT
361 TAAGACCNTC AANAAAGCAA ATCTATATAT CTTAANCAAT CCTACTTAAC AAGNCCTCAA
421 TCAAAGNCAA ATCCTACTCT GATGTTAAAA TAGTTTTGTT GGCACATGTG TAGGCTGCAA
481 GTCCTCTGTG AANCATGATT ATAGAGTCTA TTTCTCAANG CACTTTAATA CTTTCTAATT
541 GCCAGAGGGA TAAAGCACAC GGTTAGAAGC TAATNTCTCT GACCNTCAGT GCCTTCCACA
601 CCCAGCACAG GAGTACAGCC TGTGAGAGTN ATGGGNNACC ACTATTGTAC CATAGTGATC
661 TAAAATAGCT CCTTTGAGCC ANGCCNNNNG ATCCAGTTGT ATANNAAATT NTTACCCCCC
721 CCCNNANNAG AATTTTNCAN NCTTNNTATG ATTGTTGAAT NATTTGTGAA TTAAATAAGA
781 TNCNTTGAAA AAAAANAAGC
```

B 70.2: Sequencing alignment of cDNA subclone **AO1#5** with **mouse carnitine O-octanoyltransferase (Crot)** by BLAST searching against the National Center for Biotechnology Information database

```
AO1#5      : 184  aagagtaggatggtgatgtgaacntggaaggaatgttgaccttaaaggaagacctgttaa 243
                  ||||||||||||||||||||||| | |||||||||||||||| |||||||| ||||||||
Mouse Crot: 2052  aagagtaggatggtgatgtga-catggaaggaatgttgac-ttaaaggaag-cctgttaa 2108

AO1#5      : 244  tgtagggttaggagagtgatgactctactagagttattactaacncttaaagtctnnctg 303
                  |||||||||||||||||||| |||| |||||||||| |||   |||||||||| |||
Mouse Crot: 2109  tgtagggttaggagagtgatg-ctct-ctagagttatt-cta--ccttaaagtctc-ctg 2162

AO1#5      : 304  tgttnnaaaccancaatgncaaagtcagatatagtgaatagaaactatgncaatgtttaa 363
                  ||||  || ||| ||||| |||||||||||||||||||||||| ||||||| ||||||||||
Mouse Crot: 2163  tgttgcaa-cca-caatg-caaagtcagatatagtgaatag-aactatg-caatgtttaa 2217

AO1#5      : 364  gaccntcaanaaagcaaatctatatatcttaancaatcctacttaacaagncctcaatca 423
                  | | |||| |||||||||||||||||||||||| |||||||||||||||| |||||||||
Mouse Crot: 2218  g--cctcaacaaagcaaatctatatatcttaa-caatcctacttaacaag-cctcaatca 2273

AO1#5      : 424  aagncaaatcctactctgatgttaaaatagttttgttggcacatgtgtaggctgcaagtc 483
                  ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Crot: 2274  aag-caaatcctactctgatgttaaaatagttttgttggcacatgtgtaggctgcaagtc 2332

AO1#5      : 484  ctctgtgaancatgattatagagtctatttctcaangcactttaatactttctaattgcc 543
                  |||||||||| |||||||||||||||||||||||||||||| |||||||||||||||||||
Mouse Crot: 2333  ctctgtgaa-catgattatagagtctatttctcaa-gcactttaatactttctaattgcc 2390

AO1#5      : 544  agagggataaagcacacggttagaagctaatntctctgaccntcagtgccttccacaccc 603
                  ||||||||||||||||||||||||||||||||||| ||||||| | ||||||||||||||||
Mouse Crot: 2391  agagggataaagcacacggttagaagctaatttctctga-catcagtgccttccacaccc 2449

AO1#5      : 604  agcacaggagtacagcctgtgagag-tnatggg-nnaccactattgtaccatagtgatct 661
                  |||||||||||||||||||||||| | |||||   |||||||||||| ||||||||||
Mouse Crot: 2450  agcacaggagtacagcctgtgagagttcatgggaaaaccactattgtacaatagtgatct 2509

AO1#5      : 662  aaaatagctcctttgagc 679
                  ||||||||||||||||||
Mouse Crot: 2510  aaaatagctcctttgagc 2527
```

B 70.3: Summary of sequence alignment of cDNA subclone **AO1#5** with **mouse Crot**

FluoroDD gel AO (AP5 + ARP10)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AO1	+	++	+	+	700	AO1#5	720	M13 reverse	*Mus musculus* carnitine O-octanoyltransferase (Crot) (2804 bp)	e-152 461/498 (92%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 71.1: DNA sequence of cDNA subclone AO2#6 (AP5 & ARP10) using M13 forward (-20) primer

```
  1 ACCNNCACTA TACGGGCNAA TNTGGGCCCT CTAGATGCNT GCTCGAGACG GCCGCCAGTG
 61 TGATGGATAT CTGCAGAATT CGCCCTTAGC GGATAACAAT TTCACACAGG AGATCTCAGA   ARP10
121 CAAAAATCCA ACTGGACAGG CTACTCACAA AAGGTGTTAC CCACTTCGTT ATATATACTN
181 CCCTGTCNAT CCCAAAATGT CATCATTAGG GAAGCCACTA AGAGTTTGGA AATAGGGGCT
241 GGAGAGATGC CTCTGTGGTT AAGAGCATTG GCTGCTCCTC TAGAGGACCC AGGTTCAATT
301 CCCCAGTGCC CACACATCAG CTCACAACTG TCTGTAACCC AGTTCTAGGT GATTTGATAC
361 CCTCACACAG ACATACATGT AGACAGATCC ACCAATACAT ATAAAATTAA AATAAAGATT
421 TTGGAAATAA CATATTGCCA AAATGAGAAT TACTTGGTAT TATTTTGGTC TGTGGCTTCT
481 AAGATTTTTT AAAATACATG AAAAATGTAG TAAATCACTA AAATTCATTT ATCTTTCTTT
541 GAAAGANNTC AATATTTTAG TATAATAAAG AGCTGTATGT NGAGCCATCT GTGTCGGGAT
601 TTGTGNNTCC TGCTCTTTTT CAGTNCCTCT CTAGTCTATG AACTCTCATG TNCCTTAGTG
661 TCCCTATACC TTCTGGTCNA ATACAAATGC CCATNANATC CATCATGTGA AAAAAAAAAA
721 GCCNTATAGT GAGGTNTTAT NACNAGGNCC NAATCCAGCC ACNCTGGGCG GNNGTNAATA
781 NNTGNATCCN ANCCTCGGTA CCANGCTTGA NNCNNTNNNT
```

B 71.2: Sequencing alignment of cDNA subclone **AO2#6** with **mouse RNase A family 4 (Rnase4)** by BLAST searching against the National Center for Biotechnology Information database

```
AO2#6          : 111  agatctcagacaaaaatccaactggacaggctactcacaaaaggtgttacccacttcgtt 170
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Rnase4: 944  agatctcagacaaaaatccaactggacaggctactcacaaaaggtgttacccacttcgtt 1003

AO2#6          : 171  atatatactncccctgtcnatcccaaaatgtcatcattagggaagccactaagagtttgga 230
                      |||||||||  | |||  || ||||||||||||||||||||||||||||||||||||
Mouse Rnase4: 1004 atatatactttc-tgt-tattccaaaatgtcatcattagggaagccactaagagtttgga 1061

AO2#6          : 231  aataggggctggagagatgcctctgtggttaagagcattggctgctcctctagaggaccc 290
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Rnase4: 1062 aataggggctggagagatgcctctgtggttaagagcattggctgctcctctagaggaccc 1121

AO2#6          : 291  aggttcaattccccagtgcccacacatcagctcacaactgtctgtaacccagttctaggt 350
                      |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Mouse Rnase4: 1122 aggttcaattccc-agtgcccacacatcagctcacaactgtctgtaacccagttctaggt 1180

AO2#6          : 351  gatttgataccctcacacagacatacatgtagacagatccaccaatacatataaaattaa 410
                      |||||||||||||||||||||||||||||||||||||| |||||| ||||||||||||
Mouse Rnase4: 1181 gatttgataccctcacacagacatacatgtagacagatc-accaatgcatataaaattaa 1239

AO2#6          : 411  aataaagattttggaaataacatattgccaaaatgagaattacttggtattattttggtc 470
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Rnase4: 1240 aataaagattttggaaataacatattgccaaaatgagaattacttggtattattttggtc 1299

AO2#6          : 471  tgtggcttctaagatttttaaaatacatgaaaaatgtagtaaatcactaaaattcattt 530
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Rnase4: 1300 tgtggcttctaagatttttaaaatacatgaaaaatgtagtaaatcactaaaattcattt 1359

AO2#6          : 531  atctttctttgaaaga-nntcaatattttagtataataaagagctgtatgt 580
                      ||| ||||||||||||   ||||||||||||||||||||||||||||||
Mouse Rnase4: 1360 atccttctttgaaagattttcaatattttagtataataaagagctgtatgt 1410
```

B 71.3: Summary of sequence alignment of cDNA subclone **AO2#6** with **mouse Rnase4**

FluoroDD gel AO (AP5 + ARP10)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AO2	+	++	+	+	695	AO2#6	760	M13 forward (-20)	*Mus musculus* ribonuclease, RNase A family 4 (Rnase4) (1412 bp)	0 458/471 (97%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 72.1: DNA sequence of cDNA subclone AO2#6 (AP5 & ARP10) using M13 reverse primer

```
  1 AGCTATTTAG GTGACACTAT AGAATACTCA AGCTATGCAT CAAGCTTGGT ACCGAGCTCG
 61 GATCCACTAG TAACGGCCGC CAGTGTGCTG GAATTCGCCC TTGTAATACG ACTCACTATA   AP5
121 GGGCTTTTTT TTTTTTCACA TGATGGATCT AATGGCCATT TGTATTTGAC CAGAATGTAT
181 AGGGACACTA AGGGACATGA GAGTTCATAG ACTAGAGAGG AACTGAAAAA GAGCAGGAGA
241 ACACAAATCC CGACACAGAT GGCTCAACAT ACAGCTCTTT ATTATACTAA AATATTGAAA
301 ATCTTTCAAA GAAAGATAAA TGAATTTTAG TGATTTACTA CATTTTTCAT GTATTTTAAA
361 AAATCTTAGA AGCCACAGAC CAAAATAATA CCAAGTAATT CTCATTTTGG CAATATGTTA
421 TTTCCAAAAT CTTTATTTTA ATTTTATATG TATTGGTGAT CTGTCTACAT GTATGTCTGT
481 GTGAGGGTAT CAAATCACCT AGAACTGGGT TACAGACAGT TGTGAGCTGA TGTGTGGGCA
541 CTGGGAATTG AACCTGGGTC CTCTAGANGA GCAGCCCATG CTCTTAANCA CACNCGCATC
601 TCTCCAGCCC CTATTNNCAA ACTCTTAGTG GCTTCCCTAA TGATGACANT TNTGGAATAA
661 CAGANNGTAT ATATAACGAN NTGGNTAACA CCTTTNGTGA GTAGCCTGTN CAGTTGGANT
721 NNTTGTNTGA NANCCTCNTG TGTGAACNTT GTNATCCCCC TAAGNGGCAA NNTNTTGNCA
781 GATTTCNTT
```

B 72.2: Sequencing alignment of cDNA subclone **AO2#6** with **mouse RNase A family 4 (Rnase4)** by BLAST searching against the National Center for Biotechnology Information database

```
AO2#6          : 265  caacatacagctctttattatactaaaatattgaaaatctttcaaagaaagataaatgaa 324
                      ||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Mouse Rnase4: 1412  caacatacagctctttattatactaaaatattgaaaatctttcaaagaaggataaatgaa 1353

AO2#6          : 325  ttttagtgatttactacatttttcatgtattttaaaaaatcttagaagccacagaccaaa 384
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Rnase4: 1352  ttttagtgatttactacatttttcatgtattttaaaaaatcttagaagccacagaccaaa 1293

AO2#6          : 385  ataataccaagtaattctcattttggcaatatgttatttccaaaatctttatttttaattt 444
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Rnase4: 1292  ataataccaagtaattctcattttggcaatatgttatttccaaaatctttatttttaattt 1233

AO2#6          : 445  tatatgtattggtgatctgtctacatgtatgtctgtgtgagggtatcaaatcacctagaa 504
                      |||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Rnase4: 1232  tatatgcattggtgatctgtctacatgtatgtctgtgtgagggtatcaaatcacctagaa 1173

AO2#6          : 505  ctgggttacagacagttgtgagctgatgtgtgggcactgggaattgaacctgggtcctct 564
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Rnase4: 1172  ctgggttacagacagttgtgagctgatgtgtgggcactgggaattgaacctgggtcctct 1113

AO2#6          : 565  agangagcagcccatgctcttaancacacncgcatctctccagcccctattnncaaactc 624
                      ||| |||||||| |||||||||| |||| ||||||||||||||||||| |||||||
Mouse Rnase4: 1112  agaggagcagccaatgctcttaaccacagaggcatctctccagcccctatttccaaactc 1053

AO2#6          : 625  ttagtggcttccctaatgatgacanttntggaataacaganngtatatataacganntgg 684
                      |||||||||||||||||||||| || |||||||||||  |||||||||||||  |||
Mouse Rnase4: 1052  ttagtggcttccctaatgatgaca-ttttggaataacagaaagtatatataacgaagtgg 994

AO2#6          : 685  ntaacacctttngtgagtagcctgtncagttgga 718
                      |||||||||| |||||||||||| ||||||||
Mouse Rnase4: 993   gtaacacctttgtgagtagcctgtccagttgga 960
```

B 72.3: Summary of sequence alignment of cDNA subclone **AO2#6** with **mouse Rnase4**

FluoroDD gel AO (AP5 + ARP10)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AO2	+	++	+	+	695	AO2#6	760	M13 reverse	*Mus musculus* ribonuclease, RNase A family 4 (Rnase4) (1412 bp)	0 435/454 (95%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 73.1: DNA sequence of cDNA subclone AO2#8 (AP5 & ARP10) using M13 reverse primer

```
  1 AGNCNTATTT AGGTGACACT ATAGAATACN NAGACNATGN CTNCNAGNCT TGGTACCNAG
 61 ACNNNGATCA CCTAGTAACG GCCGCCAGTG TGCTGGAATT CGCCCTTGTA ATACGACTCA   AP5
121 CTATAGGGCT TTTTTTTTTT TCAAATGGAA ATGCTTTAAT TTTATATTCA CAAAATGAAT
181 TGCAACATAT CATAATGAAT TTTGCTAATA TTCTTTTCTG TGGTAGTGTA AAAATTTCTA
241 ATACAACTTG ATCCTTTTGC CTGCTCAAAG GAGCTATTTT AGATCACTAT TGTACAATAG
301 TGGTTTTCCC ATGAACTCTC ACAGGCTGTA CTCCTGTGCT GGGTGTGGAC TTTTTCTGAA
361 CCCTTCCATC TTCCTTCTGT CTCTAGGACT CTCTCATCAA CATAATGTGC AATGTTCACC
421 ATGACCATTG CATCGTAAGG AGCATGATCA CAGCAACAGC CAAATATCCC ATTAGCAAAG
481 GAAATCAAAT TATAGGACTT GTCACCCCAG CGTACTGATG GATCTCCACC AAGAAGCATT
541 TCAAAGACCT GAGAATATCC TNCTGGGGTN GCATGTGGAC TGCTATCTTC TATGGAATAC
601 ACAAATAAAC TGGTTNGAAT TTTNTCTAAT AAAGTTAAGT TCTCTGGATC NAGANCTAAT
661 CAGATATNCT CTNGCCCTTN GNCNNCCCGA GTCCGCTNCC CCCCTAGTNA NNGCNGCTAT
721 ACTGGCTCCA ACAGNTCNGT CCGACCCNNC CTGCTGCATT NTNTAACTNG NNGCACCTCN
781 GCTCTNAAAT GTTATCNNTA NNNNCNAATN NTGNNANNTC NATNNNNNNG G
```

B 73.2: Sequencing alignment of cDNA subclone **AO2#8** with **mouse carnitine O-octanoyltransferase (Crot)** by BLAST searching against the National Center for Biotechnology Information database

```
AO2#8      : 347  ggacttttctgaaccttccatcttccttctgtctctaggactctctcatcaacataat 406
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Crot: 1253  ggacttttctgaaccttccatcttccttctgtctctaggactctctcatcaacataat 1194

AO2#8      : 407  gtgcaatgttcaccatgaccattgcatcgtaaggagcatgatcacagcaacagccaaata 466
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Crot: 1193  gtgcaatgttcaccatgaccattgcatcgtaaggagcatgatcacagcaacagccaaata 1134

AO2#8      : 467  tcccattagcaaaggaaatcaaattataggacttgtcaccccagcgtactgatggatctc 526
                  | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Crot: 1133  ttccattagcaaaggaaatcaaattataggacttgtcaccccagcgtactgatggatctc 1074

AO2#8      : 527  caccaagaagcatttcaaagacctgagaatatcctnctggggtngcatgtggactgctat 586
                  |||||||||||||||||||||||||||||| || ||||||| ||||||||||||||||
Mouse Crot: 1073  caccaagaagcatttcaaagacctgagaatattcttctggggttgcatgtggactgctat 1014

AO2#8      : 587  cttctatggaatacacaaataaactggttngaattttntctaataaagttaagttctctg 646
                  |||||||||||||||||||||||||||||| ||||||| |||||||||| ||||||||||
Mouse Crot: 1013  cttctatggaatacacaaataaactggtttgaatttttctaataaagtcaagttctctg 954

AO2#8      : 647  gatcnaganctaatcagatatnctctngcc 676
                  |||| ||| |||||||||||| |||| |||
Mouse Crot: 953   gatcaaga-ctaatcagatattctcttgcc 925
```

B 73.3: Summary of sequence alignment of cDNA subclone **AO2#8** with **mouse Crot**

FluoroDD gel AO (AP5 + ARP10)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AO2	+	++	+	+	695	AO2#8	740	M13 reverse	*Mus musculus* carnitine O-octanoyltransferase (Crot) (2804 bp)	e-160 319/330 (96%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 74.1: DNA sequence of cDNA subclone AO8#2 (AP5 & ARP10) using M13 forward (-20) primer

```
  1 TCANNATAGG GGCNAATTGG GCCCTNCTAG ATGCNTGCTC GAGACGGCCG CCAGTGTGAT
 61 GGATATCTGC AGAATTCGCC CTTAGNCGGA TAACAATTTC ACACAGGAGA TCTCAGACAA   ARP10
121 AAATCCAACT GGACAGGCTA CTCACAAAAG GTGTNACCCA CTTCGTTATA TATACTTTCT
181 GTTATCNCAA AATGTCATCA TTAGGGAAGC CACTAAAGAG TNTGGGAAAT AGGGGGCTGG
241 AGAGATGCCT CTGTGGTTAA GAGCATNGNN NTGCTCCTCT AGAGNNCCCA GGTTCANNTC
301 CCAGTGCCCA CACATCAGCT CACAACTGTC TGTAACCCAG TTCTAGGTGA TTTGATACCC
361 TCACACAGNA CATACATGTA AAAAGATCAC CAATNNATAT AAAATTANAA TAAAGATTTT
421 GGGAAATGAA AAAAAAAAGC CCCTATAGTG AGNTCNTATN NCAAGGGGCN ANANNCCAGC
481 CACACTGGGC GGGCCGTTTA CTANGTGGGA TTCCGAGCCT CGGGTACCCA ACCNNGATGC
541 CATAGCCTNG AGTAANNCTA TAGTGTAACC TAAATAGCTN GGCGTANTCA TGGTNATANA
601 AGNNAAANAT NCCCCNCAAG GANNNCCGCC TCACAAATTC CCAACANNAC AATACCGAAN
661 CCNGGGANNN CACNTAAAAG NNNGTNAAAG GCCCNNNGGG GNTGNCCTNA ANNTGGANNN
721 TGAGNCCNCA ACCNCACACA NNTTAATTTG NNNGTNGCNC CCTACANTGN NCNNNNTTTC
781 CAAGTNNGGN ANCCCTGTNN TGCCCANCTG NNTNATTGAT TCGGCCACGC CCGGGANNNG
841 NNGTTGGNNN TGGNNCCTTT TCGCCTNNNC CCTANTGACT NNNNNGCCTG GGTGTTGGG
```

B 74.2: Sequencing alignment of cDNA subclone **AO8#2** with **mouse RNase A family 4 (Rnase4)** by BLAST searching against the National Center for Biotechnology Information database

```
AO8#2          : 108  agatctcagacaaaaatccaactggacaggctactcacaaaaggtgtnacccacttcgtt 167
                      ||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Mouse Rnase 4: 944  agatctcagacaaaaatccaactggacaggctactcacaaaaggtgttacccacttcgtt 1003

AO8#2          : 168  atatatactttctgttatcncaaaatgtcatcattagggaagccactaaagagtntggga 227
                      |||||||||||||||||  ||||||||||||||||||||||||||| |||||| | |||
Mouse Rnase 4: 1004 atatatactttctgttattccaaaatgtcatcattagggaagccact-aagagttt-gga 1061

AO8#2          : 228  aatagggggctggagagatgcctctgtggttaagagcatngnnntgctcctctagagnnc 287
                      |||| ||||||||||||||||||||||||||||||||||| |   ||||||||||||  |
Mouse Rnase 4: 1062 aata-ggggctggagagatgcctctgtggttaagagcattg-gctgctcctctagaggac 1119

AO8#2          : 288  ccaggttcanntcccagtgcccacacatcagctcacaactgtctgtaacccagttctagg 347
                      |||||||||  ||||||||||||||||||||||||||||||||||||||||||||||||
Mouse Rnase 4: 1120 ccaggttcaattcccagtgcccacacatcagctcacaactgtctgtaacccagttctagg 1179

AO8#2          : 348  tgatttgataccctcacacagnacatacatgtaaaaagatcaccaatnnatataaaatta 407
                      |||||||||||||||||||| |||||||||| | ||||||||||  |||||||||||
Mouse Rnase 4: 1180 tgatttgataccctcacacag-acatacatgtagacagatcaccaatgcatataaaatta 1238

AO8#2          : 408  naataaagattttgg 422
                      ||||||||||||||||
Mouse Rnase 4: 1239 aaataaagattttgg 1253
```

B 74.3: Summary of sequence alignment of cDNA subclone **AO8#2** with **mouse Rnase4**

FluoroDD gel AO (AP5 + ARP10)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AO8	+	-	+	+	400	AO8#2	400	M13 forward (-20)	*Mus musculus* ribonuclease, RNase A family 4 (Rnase4) (1412 bp)	e-121 294/315 (93%)

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 75.1: DNA sequence of cDNA subclone AP4#4 (AP12 & ARP2) using M13 forward (-20) primer

```
  1 CCNCCTATAC GGATCGCGCN NACATCGTCG CCANNNGCCC ANTGTATNAT TTNANAATCC
 61 CCTGTAATAC GACTCACTAT AGGGCTTTTT TTTTTTTCTA ATANCCTCTC TAGGTNCAGG   AP12
121 GTGTACGTAT ATATCTTATC CACATCCNNA TNCATAAATC AAGTCGACAC CGCTTATCTG
181 TAAGGTGGCT CTATTTCTCT TGTCCTTCNC NNTACNCGGG ACANNACTCN NTCAACATAC
241 ATACAACNCC GACCTGCCAT CGCTCCGGTC TGAANCTCAC ATCCACCCTN ACGACTTTAA
301 TCGTTGAACA AACAAACCAT TAATACCCTT CTACACCATT GGGATGTCCT GATCCAACCA
361 TCGAGGTNNT AAACCCTAAT TGTNATATGA ANCTCTTAAA TAGGATCGCG CTGTNATNCT
421 ANGGTANNTN GGTCGTGATC ATTTNGGGTN TANATTGTNA TNNNNTGTNT NGTNNTNNNN
481 GATTTNGTNT NTNNNNNNNN
```

B 75.2: Sequencing alignment of cDNA subclone **AP4#4** with **mouse mitochondrion** by BLAST searching against the National Center for Biotechnology Information database

```
AP4#4                 : 293  gactttaatcgttgaacaaacaaaccattaatacccttctacaccattgggatgtcctga 352
                             ||||||||||||||||||||||||||| |||||||||||| | ||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2498 gactttaatcgttgaacaaacgaaccattaatagc-ttctacaccattgggatgtcctga 2440

AP4#4                 : 353  tccaaccatcgaggtnntaaaccctaattgt-natatgaanctcttaaataggatcgcgc 411
                             ||||| |||||||||| ||||||||||||||| |||||||| |||||||||||||| ||||
Mouse mitochondrion: 2439 tccaa-catcgaggtcgtaaaccctaattgtcgatatgaa-ctcttaaataggattgcgc 2382

AP4#4                 : 412  tgt 414
                             |||
Mouse mitochondrion: 2381 tgt 2379
```

B 75.3: Summary of sequence alignment of cDNA subclone **AP4#4** with **mouse mitochondrion**

FluoroDD gel AP (AP12 + ARP2)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AP4	+	++	+	+	680	AP4#4	720	M13 forward (-20)	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	7e-30 113/123 (91%)

* Sequence of primer was not completely matched with the expected sequence.

Appendix B. DNA sequences and sequencing alignments of FluoroDD fragments

B 76.1: DNA sequence of cDNA subclone AP4#4 (AP12 & ARP2) using M13 reverse primer

```
  1 CNNNTATTTA GGTGACACTA TAGAATACTA CAAGACTATN NATNCAAGAC TTGGTACCGA
 61 GACTACGGAT CCACTAGTAA CGGCCGCCAG TGTGCTGGAA TTNCGCCCTT AGACGGATAA    ARP2
121 CAATTTCACA CAGGAGCTAG CATGGACGGC TAAACGAGGG TCCAACTGTC TACTTATCTA
181 TAATCAGTGA AATTGACCTT TNCAGTGAAG AGGCTGAAAT ATAATAATAA GACGAGAAGA
241 CCCTATGGAG CTTAAATTAT ATAACTTATC TATTTAATNT ATAAAACCTA ATGGCCCAAA
301 GACTATAGTA TAAGTTTGAA ATTTNGGTTG GGGTGACCTC GAGAATAAAA AATCNTCAGA
361 ATGATTATAA CTAATACACN NNNTNATCCT NTNTATGANA TTTTNATNNA NTCTGNATGC
421 CTTTNCANNT ANANTNNANT NNNNTNNNNN NNNNNNANAA AAAAAAAAAN NNNNNGNANN
481 NNNNNANANA ANAACAAANA AAAAAAAAAA NNCAAACAAA AAAAAAANAA CANANAACNC
541 ACNNAAAANN AAAAAAAACA CAA
```

B 76.2: Sequencing alignment of cDNA subclone **AP4#4** with **mouse mitochondrion** by BLAST searching against the National Center for Biotechnology Information database

```
AP4#4                 : 141  catggacggctaaacgagggtccaactgtctacttatctataatcagtgaaattgaccttt 200
                               |||| |||||||||||||||||||||||||||||| ||||||| ||||||||||||||||||
Mouse mitochondrion: 2065 catgaacggctaaacgagggtccaactgtct-cttatctttaatcagtgaaattgaccttt 2123

AP4#4                 : 201  tncagtgaagaggctgaaatataataataagacgagaagaccctatggagcttaaattat 260
                               | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mouse mitochondrion: 2124 t-cagtgaagaggctgaaatataataataagacgagaagaccctatggagcttaaattat 2182

AP4#4                 : 261  ataacttatctatttaatntataaaacctaatggcccaaagactatagtataagtttgaa 320
                               |||||||||||||||||| ||| ||||||||||||||||| ||||||||||||||||||
Mouse mitochondrion: 2183 ataacttatctatttaatttattaaacctaatggcccaaaaactatagtataagtttgaa 2242

AP4#4                 : 321  atttnggttggggtgacctc-gagaataaaaaatcntcagaatgattataac 371
                               |||| |||||||||||||||| |||||||||||||| || ||||||||||||
Mouse mitochondrion: 2243 atttcggttggggtgacctcggagaataaaaaatcctccgaatgattataac 2294
```

B 76.3: Summary of sequence alignment of cDNA subclone **AP4#4** with **mouse mitochondrion**

FluoroDD gel AP (AP12 + ARP2)						Subclone I.D.	Sequencing			
Fragment no.	PPARα (+/+)		PPARα (-/-)		FluoroDD fragment size (bp)		Size of insert after *Eco*RI cut (bp)	Primer	Gene homology to Blast search (Transcript size)	E-value (% Identity)
	CTL	Wy	CTL	Wy						
AP4	+	++	+	+	680	AP4#4	720	M13 reverse	*Mus musculus* clone LA9 mitochondrion, complete genome (16300 bp)	7e-95 221/232 (95%)